The Institute of Paper Chemistry

Appleton, Wisconsin

Doctor's Dissertation

The Location and Significance of the *O*-Acetyl Groups in a Glucomannan from Paraña Pine

Gerald Katz

June, 1964

THE LOCATION AND SIGNIFICANCE OF THE O-ACETYL GROUPS IN A GLUCOMANNAN FROM PARAÑA PINE

A thesis submitted by

Gerald Katz

B.S. 1959, Boston University
M.S. 1961, Lawrence College

in partial fulfillment of the requirements
of The Institute of Paper Chemistry
for the degree of Doctor of Philosophy
from Lawrence College,
Appleton, Wisconsin

June, 1964

TABLE OF CONTENTS

	Page
SUMMARY	1
INTRODUCTION	4
IDENTIFICATION OF O-ACETYL GROUPS	5
Introduction	5
Experimental	5
ISOLATION OF NATIVELY ACETYLATED GLUCOMANNAN	7
Introduction	7
O-Acetyl Groups in Wood	7
Softwood Glucomannans	8
Experimental	12
Preparation of Extractive-Free Wood	12
Isolation of Holocellulose	14
Ball-Milled Holocellulose	14
Dimethylsulfoxide Extraction	15
Results	15
LOCATION OF O-ACETYL GROUPS	21
Introduction	21
Methylation Techniques	21
Blocking Groups	22
Experimental	23
Carbanilation of Natively Acetylated Glucomannan	23
Methylation of O-Acetyl Glucomannan Phenylcarbamate	24
Lithium Aluminum Hydride Reduction of O-Methyl Glucomannan N-Methyl-Phenylcarbamate	26
Characterization of O-Methyl Glucomannan	28
Hydrolysis of O-Methyl Glucomannan	28

Isolation of Methylated Sugars 29

Ratio of Methylated Sugars 31

Identification of Methylated Sugars 31

Summary and Discussion of Reaction Sequence Employed for Location of O-Acetyl Groups 33

CHARACTERIZATION OF NATIVELY ACETYLATED GLUCOMANNAN 41

Infrared Spectra 41

Optical Rotation 43

Intrinsic Viscosity 44

EFFECT OF THE O-ACETYL GROUPS ON THE PHYSICAL PROPERTIES OF THE ACETYLATED GLUCOMANNAN 46

Introduction 46

Experimental 48

Polarization Microscopy 48

X-ray Diffraction 52

Electron Microscopy 56

Solubility 59

DISCUSSION OF RESULTS 60

ACKNOWLEDGMENTS 66

LITERATURE CITED 67

APPENDIX I. GLOSSARY OF ABBREVIATIONS 73

APPENDIX II. GENERAL METHODS OF ANALYSIS 74

APPENDIX III. PRELIMINARY EXPERIMENTS IN THE PREPARATION OF THE O-ACETYL GLUCOMANNAN PHENYLCARBAMATE 79

APPENDIX IV. ISOLATION AND CHARACTERIZATION OF GLAZIER'S ALKALI-EXTRACTED PARAÑÁ PINE GLUCOMANNAN 80

APPENDIX V. ATTEMPTED FRACTIONATION OF ACETYLATED GLUCOMANNAN ON DEAE CELLULOSE 81

SUMMARY

The general aim of this thesis was to obtain information regarding the constitution of wood components as they exist in their native state. The component chosen for study was the natively acetylated glucomannan of Paraña pine [Araucaria angustifolia (Bert) Kuntze].

Specific goals included locating the O-acetyl groups among the component sugars in the natively acetylated glucomannan, and characterization of the acetylated polymer with emphasis on the effects of the O-acetyl groups on the polymer's physical properties.

The natively acetylated glucomannan was obtained from the dimethylsulfoxide extract of a ball-milled Paraña pine chlorite holocellulose. The polymer had 5.86% O-acetyl, an intrinsic viscosity of 0.38 dl./g. in cupriethylenediamine, and an $[\alpha]_D$ of -28.7° in water. Galactose, glucose, and mannose were present in the ratio of 0.34:1:3.8. The O-acetyl and sugar contents of this polymer were similar to those of other natively acetylated glucomannans (24, 27). The analyses of the acetylated polymer indicated that it was similar to the deacetylated glucomannan obtained on alkaline extraction of this wood, except for the presence of the O-acetyl groups. Certain properties of the acetylated glucomannan were similar to alkali-extracted softwood glucomannans.

The O-acetyl groups in the acetylated glucomannan were located by a series of blocking and substitution reactions in which the labile O-acetyl groups were replaced by O-methyl groups. First, the acetylated polymer was swollen in dimethylformamide and treated with an excess of phenylisocyanate to convert the free hydroxyls to phenylcarbamoyl groups.

Subsequent methylation of the O-acetyl glucomannan phenylcarbamate by the Kuhn procedure (78) replaced the O-acetyl groups with O-methyl groups, and N-methylated the phenylcarbamoyl groups all in one step, resulting in the direct formation of the O-methyl glucomannan N-methyl-phenylcarbamate. This one-step deacetylation and subsequent methylation of the resulting free hydroxyls, observed in this study, was unique in that these reactions would normally be carried out in two steps.

Finally, the N-methyl-phenylcarbamoyl blocking groups were removed by reduction with lithium aluminum hydride in tetrahydrofuran to yield an O-methyl glucomannan which had methoxyl groups in the sites of the original O-acetyl groups. Unfortunately, some demethylation occurred during the preparation of the O-methyl glucomannan. However, the available literature indicated that the loss of methoxyl groups could be assumed to have occurred at random, so that the location of the remaining O-methyl groups could still be determined.

To locate the positions of attachment of the methoxyl groups the O-methyl glucomannan was hydrolyzed, and the methylated sugars were resolved by preparative paper chromatography. The only methylated sugars found were 3-O-methyl-D-glucose and 3-O-methyl-D-mannose. From the ratio of these sugars, it was determined that in the natively acetylated glucomannan, 15.6% of the mannose units and 6.4% of the glucose units carried O-acetyl groups in the 3-position.

The effects of the O-acetyl groups on the physical properties of the acetylated glucomannan were studied by polarization microscopy, x-ray diffraction, and electron microscopy. The results indicated that the glucomannan may be amorphous in its native state and remains in this condition even after

isolation as long as the O-acetyl groups remain intact. Apparently, the O-acetyl groups in the 3-position of the mannose and the glucose units are capable of restricting the glucomannan molecules to randomly oriented configurations, due in part to the bulkiness of the O-acetyl groups relative to the hydroxyl groups. The initial effect of removing these groups is believed to be an increase in intermolecular orientation followed by a gradual time-dependent development of lateral order through intermolecular hydrogen bonding. In support of this theory, it was observed that the acetylated glucomannan isolated in this work, remained water soluble, appeared isotropic to polarized light, and gave a diffuse x-ray diffractogram even after aging for over a year. Alkali-extracted (deacetylated) glucomannans, on the other hand, become insoluble in alkali, show birefringence under polarized light, and give x-ray diffractograms indicative of ordered structures on aging after isolation.

The importance of the O-acetyl groups in the stabilization of the glucomannan during the bisulfite-acid sulfite pulping of softwoods is also discussed.

The O-acetyl groups in the Paraña pine used as a source of the acetylated glucomannan were positively identified by the preparation of p-nitrobenzyl acetate and p-bromophenacyl acetate.

INTRODUCTION

An important question often overlooked in the study of wood polysaccharides is how well the isolated hemicellulose represents the polysaccharide as it existed in the wood prior to extraction. Unfortunately, the majority of the methods currently employed for the isolation and purification of hemicelluloses require the use of alkaline solutions. Under these conditions the polysaccharides are not only somewhat degraded, but any partially acetylated hemicelluloses are de-esterified. The use of alkaline solutions can be circumvented, however, by using dimethylsulfoxide. This neutral solvent can extract polysaccharides which still contain their original O-acetyl groups, from both holocelluloses and acid sulfite pulps (1). Characterization of these presumably unaltered hemicelluloses may provide a better understanding of their function in fiber development and of their subsequent modification during pulping.

The steps employed in this study included the isolation of a natively acetylated glucomannan from Paraña pinewood, chosen because of its relatively high mannose and low xylose contents, the location of the O-acetyl groups among the component sugars within the polymer, and finally, characterization of the polymer with specific emphasis on the effects of the O-acetyl groups on the polymer's physical properties.

IDENTIFICATION OF O-ACETYL GROUPS

INTRODUCTION

In addition to O-acetyl groups, the early studies of the acyl groups in wood indicated that significant amounts of formyl groups (10 to 20% of the volatile acids) were also present (2, 3). However, later work by Timell (4) showed that untreated wood contained few, if any, formyl ester groups (0.01 to 0.02%); and that the formic acid previously obtained was an artifact resulting from the decomposition of the wood polysaccharides under the test conditions. The ester groups in extractive-free wood (excluding pectin) were shown to be predominantly O-acetyl groups. They amounted to 1% in softwoods and 3 to 5% in temperate zone hardwoods.

EXPERIMENTAL

About 10 g. of extractive-free Paraña pinewood meal were dried *in vacuo* at 40°C. and placed in the acetyl apparatus (5) along with 100 ml. of dry methanol. After refluxing for 30 minutes, 25 ml. of 0.2N sodium methoxide were added and the methyl acetate distilled. Two 30-ml. portions of methanol were added, and each in turn also distilled. To saponify the methyl acetate, the distillate was refluxed with 50 ml. of 0.1N sodium hydroxide for 15 minutes. The excess alkali was neutralized to phenolphthalein with 0.1N hydrochloric acid and then a slight excess of acid was added. The solution was evaporated to about 3 ml. followed by the addition of a few more drops of acid. Then 278 mg. of p-nitrobenzyl bromide and 8 ml. of methanol were added. After refluxing for two hours, distilled water was added until a cloudiness appeared. The crystals that soon formed were filtered and washed once with 4 ml. of 5% sodium

carbonate and then twice with 2 ml. of cold water. The product was recrystallized from hot methanol and washed twice with 50% aqueous methanol. The m.p. and mixed m.p. with authentic p-nitrobenzyl acetate was 77-78°C.

The p-bromophenacyl ester was prepared in a similar manner. It had a m.p. and mixed m.p. of 84-85°C. with authentic p-bromophenacyl acetate.

The acyl groups in Paraña pinewood are thus positively identified as O-acetyl groups through the preparation of the aforementioned derivatives.

ISOLATION OF NATIVELY ACETYLATED GLUCOMANNAN

INTRODUCTION

O-ACETYL GROUPS IN WOOD

The O-acetyl groups in wood were first believed to be associated with the lignin (6-9), but this concept was abandoned when suitable techniques were developed for the isolation of holocelluloses. Thus, both hardwood and softwood holocelluloses contained nearly all the O-acetyl groups originally present in the wood (10-14).

Further localization of the O-acetyl groups within the holocellulose still presented a formidable problem because the alkaline solutions commonly used for extraction of hemicelluloses saponified the O-acetyl groups. Consequently, only hot water extractions could be employed, and from hardwoods acetylated hemicelluloses were obtained containing xylose and uronic anhydride (15-18).

A significant breakthrough in this area was the discovery of dimethylsulfoxide as a solvent for natively acetylated hemicelluloses (1). The studies of hardwoods showed that dimethylsulfoxide extracted partially acetylated 4-O-methylglucuronoxylans (9 to 17% O-acetyl) from holocelluloses (19-22) and from sulfite pulps (23). The molecular weight of the partially acetylated polymer isolated by Timell (21) from white birch indicated that it was similar to that obtained on alkaline extraction of this wood except for the presence of the O-acetyl groups. Thus, in the hardwoods the O-acetyl groups are apparently associated with the 4-O-methylglucuronoxylan.

Investigation of the softwood O-acetyl groups proved to be more difficult than for the hardwoods. The isolation of natively acetylated polysaccharides

from softwoods appeared to be greatly dependent on alteration of the fine structure of the holocellulose prior to extraction with neutral solvents such as dimethylsulfoxide or hot water. Some of the methods used to increase the accessibility of the acetylated polysaccharides included treatment in a Valley beater (24) or ball mill (25); dry grinding (26) and grinding in toluene (27) in a vibratory ball mill; and swelling in liquid ammonia (28)*. When the holocellulose was modified by one of these pretreatments, partially acetylated glucomannans could be extracted by hot water or dimethylsulfoxide. The O-acetyl contents of the purified glucomannans obtained by Meier (24) and Annergren, et al. (27) when based on the wood, were in good agreement with the directly analyzed O-acetyl contents of the wood. Thus, the evidence presently available indicates that the softwood O-acetyl groups are associated with the glucomannan portion of the wood.

SOFTWOOD GLUCOMANNANS

The predominant noncellulosic carbohydrates in softwoods have been shown to be heteropolymers containing mannose and glucose in proportions ranging from 2.5:1 to 4.0:1. Small amounts of galactose have also been found in certain of these polymers depending on their source. The galactose units appear to be present as terminal nonreducing end groups linked to the main chain by α 1$\rightarrow$6 glycosidic bonds (35, 42).

Alkali-extracted (deacetylated) glucomannans have shown a striking similarity in molecular structure. As indicated by the investigations summarized in

*Exposure to liquid ammonia was very short (30 seconds) so that only the physical effect of the anhydrous ammonia would be effective and not the chemical effects which would include deacetylation.

Table I (29-54) these polysaccharides appeared to be essentially linear macromolecules of D-glucose and D-mannose linked by β 1→ 4 glycosidic bonds. The results of linkage analysis, methylation studies, and periodate oxidation followed by degradation of the oxidized polysaccharide by F. Smith's procedure (borohydride reduction followed by hydrolysis) (55), were all in accord with the schematic structure shown in Fig. 1. The possible existence of limited branching as well as the significance of the galactose residues are still unresolved questions. Additional information regarding alkali-extracted softwood glucomannans may be obtained from recent reviews by Hirst (56) and Aspinall (57).

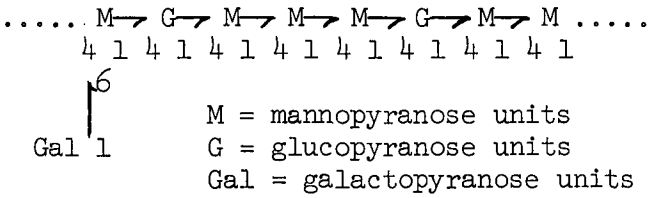


Figure 1. Schematic Representation of an Alkali-Extracted Softwood Glucomannan from Holocelluloses and Kraft Pulps

Natively acetylated softwood glucomannans, in contrast to those extracted by alkali, have been isolated only recently and the results of these investigations are summarized in Table II. Hägglund, et al. (1) found an acetylated glucomannan in the hot water extract of a spruce chlorite holocellulose after pre-extraction with dimethylsulfoxide. Koshijima (25) extracted a ball-milled pine chlorite holocellulose with acidified dimethylsulfoxide–boric acid solutions and isolated four acetylated glucomannan fractions. The polymers obtained by both Hägglund, et al. (1) and Koshijima (25) contained considerable xylose and uronic acid units and were not purified further.

After treating a spruce chlorite holocellulose for one hour in toluene in a vibratory ball mill, Annergren, et al. (27) obtained an acetylated glucomannan

TABLE I

ALKALI-EXTRACTED SOFTWOOD GLUCOMANNANS[a]

Species	$[\alpha]_D$, degrees[b]	$[\eta]$, dl./g.[c]	$\overline{DP}_n$[g]	Molar Ratio Gal.	Gl.	Man.
Tsuga						
Western hemlock[d] (29,30)	-35	0.22	--	0.00	1	3.0
Western hemlock (31,32)	-35	0.56	--	0.10	1	3.3
Eastern hemlock (33)	-40	--	--	0.15	1	3.0
Pinus						
Southern pine[d] (31)	--	--	--	0.00	1	3.0
Scots pine (34)	-31	--	93	0.14		3.4
Jack pine (35)	-26	--	20	0.11	1	2.9
White pine (36)	-32	--	95	0.15	1	3.8
Loblolly pine (37-39)	-20	--	--	0.15	1	2.7
Picea						
Norway spruce[d] (40-42)	-39	--	68-100	present	1	3.5-4.0
Norway spruce (43)	--	--	--	0.00	1	3.5
White spruce (44,45)	-34	0.24	107	0.12	1	3.1
White spruce[e] (46)	-32	0.34	70	0.05	1	3.2
Black spruce[d] (47)	--	0.43	--	0.18	1	3.9
Black spruce[f] (47)	--	0.25	--	0.00	1	3.2
Black spruce (47)	--	0.34	--	0.11	1	3.9
Englemann spruce (48)	--	--	--	0.20	1	3.0
Sitka spruce (49)	-33	--	--	present	1	2.5
Spruce[d] (50)	-44	--	--	0.00	1	4.6
Thuja						
Western red cedar (51)	-37	--	--	0.05	1	2.5
Ginkgo						
Ginkgo biloba (52)	-36	--	96	0.19	1	3.6
Abies						
Amabalis fir (53)	-40	--	--	0.10	1	3.0
Araucaria						
Paraña pine (54)	-36	0.41	--	0.08	1	2.6

[a]Glucomannans were extracted from holocelluloses unless otherwise noted.

[b]Optical rotation in aqueous alkali.

[c]Intrinsic viscosity in cupriethylenediamine.

[d]Obtained from a sulfite pulp.

[e]Obtained from a bisulfite-acid sulfite pulp.

[f]Obtained from a kraft pulp.

[g]Number average degree of polymerization.

TABLE II

NATIVELY ACETYLATED SOFTWOOD GLUCOMANNANS

Source	Carbohydrates, %[a]					O-Acetyl, %[b]	Uronic Acid, %[b]
	Gal.	Gl.	Man.	Ar.	Xy.		
Spruce (1) (*Picea excelsa*)	--	17.7[e]	66.4	1.5	14.4	10.0	12.6
Akamatsu (25) (*Pinus densiflora*)							
Fraction 1	--	13.7	21.6	--	64.7	4.22	15.7
Fraction 2	--	20.5	40.2	--	39.3	6.68	10.3
Fraction 3	--	28.4	36.8	--	34.8	3.97	--
Fraction 4	--	26.3	46.8	--	26.9	6.55	11.5
Spruce (27) (*Picea abies*)							
Fraction II	4.7	17.9	68.2	2.0	7.2	5.4	--
Fraction II_p[c]	1.3	20.9	77.8	--	--	7.9	--
Pine (24) (*Pinus silvestris*)							
Fraction B	8.4	16.5	60.8	2.8	11.5	4.51	--
Fraction B_p[d]	4.8	18.0	77.2	--	--	5.95	--

[a] Carbohydrate content adjusted to 100% of material analyzed.

[b] Based on material analyzed.

[c] Fraction II after purification by DEAE cellulose chromatography.

[d] Fraction B after purification by DEAE cellulose chromatography.

[e] Also includes galactose.

by extraction with dimethylsulfoxide. Meier (24) treated a Scots pine chlorite holocellulose for one hour in a Valley beater. After extraction with dimethylsulfoxide, an acetylated glucomannan was obtained in the subsequent hot water extract after precipitation of a contaminating 4-O-methyl-glucuronoarabinoxylan with cetyl-trimethylammonium bromide. Meier (24) and Annergren, et al. (27) purified their acetylated glucomannans by diethylaminoethyl (DEAE)* cellulose chromatography. The small amounts of xylose and arabinose units were removed, and the purified acetylated glucomannans contained only galactose, glucose, and mannose units.

The method chosen in this work for the isolation of a natively acetylated glucomannan involved extraction of a chlorite holocellulose with dimethylsulfoxide after pretreatment in an Abbé pebble mill.

EXPERIMENTAL

PREPARATION OF EXTRACTIVE-FREE WOOD

Two bolts of Paraña pinewood [Araucaria angustifolia (Bert) Kuntze] were obtained from Lutcher S. A. Sao Paulo, Brazil. They were 26 and 52 years old and contained mostly sapwood. The bark was removed and the bolts reduced to chips, which were passed through a 12-inch Sprout-Waldron refiner with coarse plates. Reduction to wood meal was obtained with a No. 1 Wiley mill using a No. 5 screen of 0.04-inch diameter openings.

The resulting wood meal was extracted twice with 70% ethanol (1 liter/100 g.), washed with methanol, and air dried. The extractive-free wood meal was analyzed for Klason lignin (58), sulfated ash (59), O-acetyl (5), and carbohydrates (60). The analyses of all materials are given in Table III.

*A glossary of abbreviations will be found on page 73.

TABLE III

ANALYSIS OF MATERIALS

Material	Yield, %[a]	Klason Lignin, %[b]	Sulfated Ash, %[b]	O-Acetyl, %[b]	Carbohydrates, %[c]				
					Gal.	Gl.	Man.	Ar.	Xy.
Original wood	100	28.8	0.33	1.35	2.4	49.4	11.6	1.7	4.4
Holocellulose	80.3	7.1	3.11	1.57	2.1	63.5	14.6	1.9	6.1
Ball-milled holocellulose	79.4	--	3.46	1.64	--	--	--	--	--
Acetylated glucomannan	1.5	--	0.73	5.86	6.1	18.1	68.7	1.7	5.4
Residue after extraction	--	--	--	1.26	2.5	64.3	13.7	1.9	5.7

[a] Based on extractive-free wood.

[b] Based on material analyzed.

[c] Carbohydrate content adjusted to 100% of material analyzed, less Klason lignin, sulfated ash, and O-acetyl.

ISOLATION OF HOLOCELLULOSE

Extractive-free wood meal (573 g.) was chlorited for 10 hours at 65°C. in a 5-gallon Pfaudler mixer. The wood meal was added to the preheated buffer solution (5 g./160 ml.) containing sodium orthophosphate ($Na_2HPO_4.12\ H_2O$, 31.6 g./l.) and citric acid ($H_3C_6H_5O_7.H_2O$, 11.7 g./l.) of pH 4.4. Then sodium chlorite (1.5 g./5 g. of wood meal) was added. Additional sodium chlorite was added every hour for nine hours, and the final pH was 5.6. The resulting holocellulose was washed with cold water, 95% ethanol, methanol and then air dried. The entire process was repeated three times with similar charges of wood meal to yield 1846 g. (80.3%) of holocellulose.

BALL-MILLED HOLOCELLULOSE

The holocellulose was ball milled in toluene in an Abbé pebble mill, consisting of a porcelain-lined jar having an internal diameter of 10.6 inches and a depth of 13.5 inches. It was mounted in a framework so as to rotate horizontally about its axis at 60 r.p.m. In a typical experiment 100 g. of holocellulose were first dried overnight in vacuo over phosphorus pentoxide at room temperature, and then added to the pebble mill along with 2300 ml. of toluene (4.75% consistency). The air was replaced with nitrogen and the mill rotated for 10 hours. At the end of the run the toluene was drained on a Büchner funnel and the ball-milled holocellulose washed with methanol and air dried. A total of eighteen 100-g. runs were made and 1777 g. of ball-milled holocellulose were obtained. The yield of ball-milled holocellulose was 79.4% based on the extractive-free wood.

DIMETHYLSULFOXIDE EXTRACTION

Ball-milled holocellulose (1600 g.) was placed in a 40-liter glass chromatography jar and extracted for 18 hours with 25 liters of dimethylsulfoxide (m.p. 18.6°C., 99.9% purity) under nitrogen. The extract (12 liters) was acidified with 75 ml. of 10% hydrochloric acid and the hemicelluloses precipitated with four volumes of methanol. A second and third extraction with 12 liters of dimethylsulfoxide yielded 12 and 14 liters of extract which were acidified and precipitated in the same manner. The precipitate was allowed to stand in methanol overnight. The following day it was transferred to a sintered-glass funnel and washed with methanol for at least two days to insure complete removal of the dimethylsulfoxide. Subsequent washings included ether and petroleum ether (30-60°C.). Before all the petroleum ether had drained off, the hemicellulose was transferred to a vacuum desiccator and dried over phosphorus pentoxide. After further drying in vacuo at 35°C. over phosphorus pentoxide, the natively acetylated Paraña pine glucomannan (GM-OAc) was obtained as a fine white powder (30 g.). The yield was 1.5% based on the extractive-free wood. The polymer was freely soluble in water even after a year's storage.

RESULTS

The isolation of the natively acetylated glucomannan is summarized in Fig. 2. A holocellulose was isolated by a modified chlorite method using citric acid–sodium phosphate buffer of pH 4.4. The isolation of the holocellulose using this buffer system had the advantage of avoiding the use of acetic acid as required by the conventional chlorite method (61). Any residual acetic acid could have interfered with subsequent O-acetyl analyses. The delignification

was quite mild, and the holocellulose contained 93.3% of the O-acetyl groups originally present in the wood.

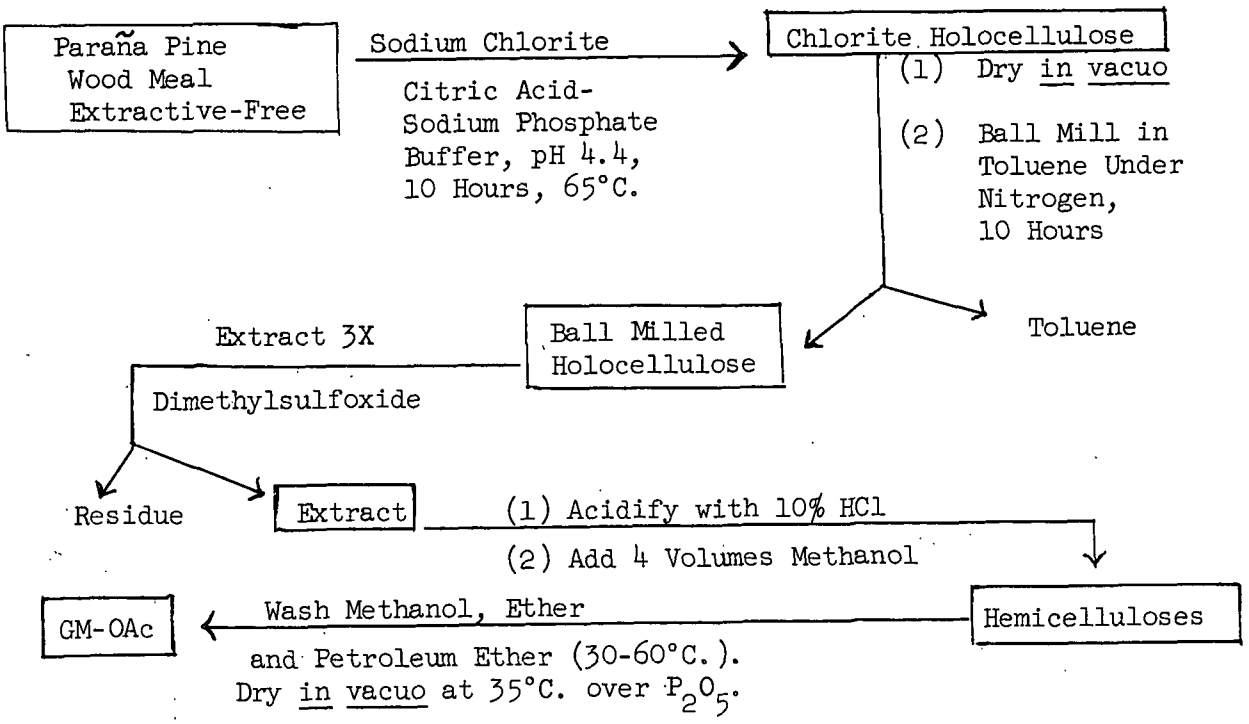


Figure 2. Flow Chart for Isolation of Natively Acetylated Paraña Pine Glucomannan (GM-OAc)

One of the major problems encountered in studying natively acetylated glucomannans is that they are not easily extracted from a conventional chlorite holocellulose (1, 62). This difficulty is probably due to the inaccessibility of the glucomannan as a result of its location within the fiber. The glucomannan content has been shown to increase steadily from the outer to the inner parts of the cell wall, and is most heavily concentrated in the S_2 layer of the secondary wall (63).

The accessibility of the acetylated polymer to extraction by neutral solvents may be improved somewhat by suitable prior modification of the

holocellulose. The two types of modification that have been employed include mechanical attrition (24-27) and decrystallization by preswelling in liquid ammonia (28). In all cases it was found that the pretreatment significantly increased the accessibility of the acetylated glucomannan to extraction by hot water or dimethylsulfoxide.

The method chosen for increasing the accessibility of the acetylated glucomannan in this work was adapted from Annergren, et al. (27). The holocellulose was ball milled for 10 hours in toluene under a nitrogen atmosphere in an Abbé pebble mill. Examination of the ball-milled holocellulose with the light microscope showed that most of the fibrous structure had been destroyed. The conditions during milling were not believed to be severe enough to cause any significant degradation in the form of depolymerization (64). This hypothesis was substantiated by the significantly high cupriethylenediamine intrinsic viscosity obtained for the acetylated glucomannan, a point which will be treated at greater length in a subsequent section (p. 45).

The acetylated glucomannan was isolated from the ball-milled holocellulose by extraction with dimethylsulfoxide. The carbohydrate and O-acetyl contents of the acetylated polymer obtained in this work were similar to Fraction B obtained by Meier (24) and Fraction II obtained by Annergren, et al. (27) as shown in Table IV.

The yield of acetylated glucomannan, O-acetyl groups, and theoretically available glucomannan (Table V) were similar to those obtained by Meier (24), although his method of isolation was quite different. As previously indicated, the method of isolation used in this work was similar to that employed by Annergren, et al. (27). The lower yield obtained in this work was probably

due to the appreciable Klason lignin content of the holocellulose (7.1%) and the fact that the Abbé pebble mill was probably not as efficient as the vibratory ball mill in increasing the accessibility of the acetylated polymer to extraction by dimethylsulfoxide. The yield of acetylated glucomannan might have been higher if the holocellulose had been delignified to about 1% Klason lignin and the ball milling time extended further (27).

TABLE IV

COMPARISON OF NATIVELY ACETYLATED GLUCOMANNANS

Source	Carbohydrates, %[a]					O-Acetyl, %[b]
	Gal.	Gl.	Man.	Ar.	Xy.	
Scots pine (24) Fraction B	8.4	16.5	60.8	2.8	11.5	4.51
Norwegian spruce (27) Fraction II	4.7	17.9	68.2	2.0	7.2	5.40
Paraña pine (this work)	6.1	18.1	68.7	1.7	5.4	5.86

[a]Carbohydrate content adjusted to 100% of material analyzed.
[b]Based on material analyzed.

TABLE V

YIELD ANALYSES OF NATIVELY ACETYLATED GLUCOMANNANS

Source	Yield of Polymer, %[a]	Yield of O-Acetyl, %[b]	Yield of Available Glucomannan, %[c,d]
Scots pine (24) Fraction B	1.35	7.2	8.4
Norwegian spruce (27) Fraction II	6.60	27.0	37.0
Paraña pine (this work)	1.50	6.4	10.0

[a]Based on the extractive-free wood.
[b]Based on the O-acetyl content of the extractive-free wood.
[c]Based on the calculated glucomannan content of the extractive-free wood.
[d]Not corrected for an unknown amount of galactoglucomannan which may vary from 1 to 4% of the extractive-free wood (65).

Although the acetylated polymer represented only 10% of the available glucomannan (1.5% of the weight of the wood), this yield was comparable to those of glucomannans obtained by alkaline extraction of holocelluloses shown in Table VI. Any alkaline extraction sequence would naturally be expected to result in higher yields of glucomannan since the high alkali concentrations commonly employed are capable of penetrating intracrystalline regions, whereas dimethylsulfoxide can only penetrate intercrystalline regions (1).

TABLE VI

YIELDS OF GLUCOMANNANS EXTRACTED FROM SOFTWOOD HOLOCELLULOSES BY ALKALI[a]

Source	Yield, %[b]	Yield Based on 15% Available Glucomannan, %[b,c]
Western red cedar (51)	4.3	28.7
Eastern hemlock (33)	6.2	41.3
Scots pine (62)	1.1	7.3
Englemann spruce (48)	8.1	54.0
Jack pine (35)	3.6	24.0
Paraña pine (54)	2.4	16.0

[a]Holocellulose was in the form of wood meal.
[b]Based on extractive-free wood.
[c]Not corrected for an unknown amount of galactoglucomannan which may vary from 1 to 4% of the extractive-free wood (65).

The majority of the original O-acetyl groups (79.1%) could be accounted for by an O-acetyl balance carried out for the isolation of the natively acetylated glucomannan. The 20.9% of the groups that were unaccounted for may have been lost through some type of fractionation during the dimethylsulfoxide extraction. Any loss of O-acetyl groups due to deacetylation by the dimethylsulfoxide was unlikely (66).

It would have been desirable to determine whether the O-acetyl content of the acetylated polymer based on the wood, corresponded to the value obtained on direct analysis of the wood. According to the work of Meier (24) and Annergren, et al. (27) these values were in agreement only after the polymer had been purified by DEAE cellulose chromatography. The results of an attempted DEAE cellulose purification of the acetylated glucomannan obtained in this work were inconclusive (Appendix V), so that an accurate O-acetyl balance of this type could not be made.

LOCATION OF O-ACETYL GROUPS

INTRODUCTION

The problem of determining the exact position of attachment of O-acetyl groups in a natively acetylated polysaccharide is generally considered quite difficult. Firstly, O-acetyl groups are sufficiently sensitive to acid and alkaline hydrolysis to necessitate restricting the experimental methods to those that can be carried out under nearly neutral conditions. Secondly, O-acetyl, as well as acyl groups in general, may migrate under the proper spatial relationships via a postulated orthoester intermediate (67, 68).

The only studies of the location of the O-acetyl groups in natively acetylated glucomannans employed periodate oxidation. Both Meier (24) and Koshijima (25) found that their natively acetylated glucomannans contained appreciable amounts of mannose following periodate oxidation. When the oxidation was performed on the corresponding deacetylated polymer, very little mannose was left unoxidized. The periodate oxidation data indicated that the O-acetyl groups were attached to the 2- or 3-position of the mannose units; and that few, if any, mannose units carried two O-acetyl groups, or an O-acetyl in the 6-position. However, it was not possible to determine whether the O-acetyl groups were attached preferentially or distributed between the 2- and 3-position.

METHYLATION TECHNIQUES

The first attempts to locate O-acetyl groups in a natively acetylated polysaccharide were concerned with the O-acetyl-4-O-methylglucuronoxylan (19-21). The polymer was methylated directly with methyl iodide and silver oxide in

dimethylformamide. Another sample was first deacetylated and similarly methylated. In theory, a comparison of the methylated sugars obtained on hydrolysis of the two methylated polysaccharides should have revealed the location of the O-acetyl groups in the original polymer. The results indicated that the O-acetyl groups were attached to the xylose residues mainly in the 3-position and to a lesser extent in the 2-position. However, this procedure was inadequate for an unambiguous location of the O-acetyl groups because 30% of them were hydrolyzed and replaced by O-methyl groups during the methylation.

BLOCKING GROUPS

A more reliable method for locating O-acetyl groups in partially acetylated polysaccharides requires the introduction of a stable blocking group on the free hydroxyls. The polymer is deacetylated and then methylated. Subsequent removal of the blocking group affords a partially methylated derivative with O-methyl groups occupying the sites of the original O-acetyl groups.

The use of phenylcarbamoyl blocking groups (69-72) (produced by the reaction of phenylisocyanate with hydroxyl groups) for the location of O-acetyl groups in partially acetylated polysaccharides has been studied on a series of glucose (73) and xylose (66) derivatives. The method was found to satisfy the following requirements: (1) Phenylisocyanate reacted quantitatively with the free hydroxyl groups under conditions that did not affect the O-acetyl groups; (2) the resulting phenylcarbamoyl groups were not affected by subsequent deacetylation; (3) the phenylcarbamoyl groups did not migrate on methylation of the deacetylated product; and (4) the phenylcarbamoyl groups were easily removed by reduction with lithium aluminum hydride to give a partially methylated product containing O-methyl groups in the sites of the original O-acetyl groups.

(66, 73). This method was used by Bouveng (20) to locate the O-acetyl groups in a natively acetylated 4-O-methylglucuronoxylan from white birch.

It appeared that the O-acetyl groups in the natively acetylated Paraña pine glucomannan could be located by using phenylcarbamoyl blocking groups and then replacing the O-acetyl groups with O-methyl groups.

EXPERIMENTAL

CARBANILATION OF NATIVELY ACETYLATED GLUCOMANNAN

After drying *in vacuo* over phosphorus pentoxide at 40°C.[1], 5.6 g. of the acetylated glucomannan were added in small portions to 110 ml. of dry dimethylformamide[2] in a 250-ml. round-bottom flask. The resulting swollen mass was agitated by hand for 15 minutes and then 15 ml. of phenylisocyanate (b.p. 60-62°C. at 20 mm.) were added. All of these operations were carried out in a dry box at about 5% relative humidity. The flask was equipped with a reflux condenser and a calcium chloride tube and placed on the steam bath. In 15 minutes the swollen mass was transformed into a clear brown solution. After six hours the solution was cooled and poured into 10 volumes of absolute ethanol. The tan precipitate gradually darkened and agglomerated into a brown gum. The supernatant liquid was decanted and centrifuged, and the combined precipitates were washed on a sintered-glass filter with absolute ethanol, ether, and petroleum ether (30-60°C.). During the washing the gum hardened and was finally obtained as a coarse tan powder. An additional 6.0 g. of the acetylated glucomannan were treated in a similar manner with 18 ml. of phenylisocyanate. A total of

---

[1] Polymers were always dried in this manner.

[2] Dimethylformamide was dried by distilling from potassium hydroxide at water pump pressure.

23.5 g. (62.5%) of O-acetyl glucomannan phenylcarbamate (GM-OAc-PC) were obtained. Anal. calcd. for $C_6H_7O_2(OCOCH_3)_{0.23}(OCONHPh)_{2.77}$: CH_3CO, 1.12; N, 7.74. Found: CH_3CO, 1.03; N, 7.52.*

The infrared spectrum of the GM-OAc-PC was characteristic of a monosubstituted urethan, Fig. 3. Absorption bands corresponding to the joint ester-amide carbonyl at 5.80 μ, the amide II grouping at 6.55 μ, and the N-H stretch at 3.05 μ were all present (74).

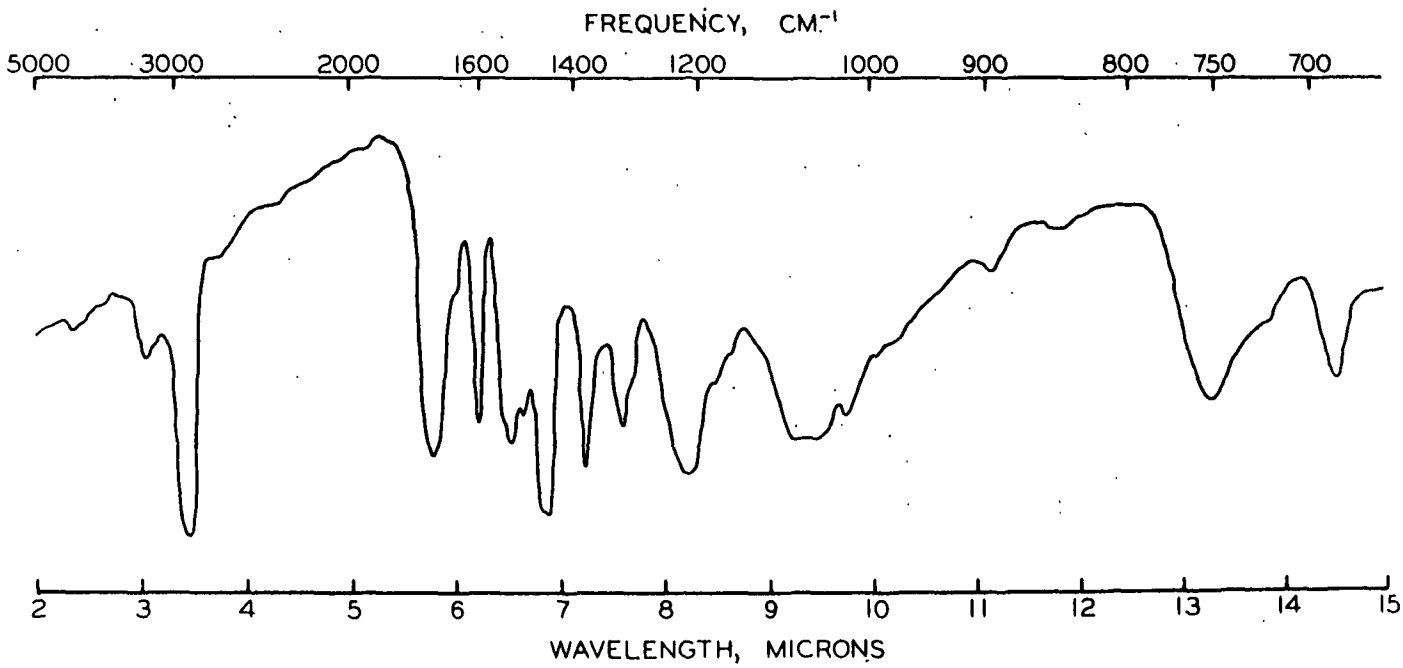


Figure 3. Infrared Spectrum of O-Acetyl Glucomannan Phenylcarbamate (Phase-Nujol)

METHYLATION OF O-ACETYL GLUCOMANNAN PHENYLCARBAMATE

Dry GM-OAc-PC (10.0 g.) was dissolved in 100 ml. of dry dimethylformamide in a 250-ml. round-bottom flask. The solution was stirred magnetically while

*The O-acetyl content of the acetylated glucomannan (5.86%) corresponds to a degree of substitution of 0.23. Subsequent elemental and group analyses were calculated on this basis.

26.0 ml. of methyl iodide (b.p. 42-44°C.) were added. Then 28.0 g. of dry, freshly prepared silver oxide* were added in five equal portions over a period of four hours. After stirring for 20 hours the reaction mixture was centrifuged and the silver residues washed with dimethylformamide. The product was isolated by pouring the dimethylformamide solution into 1200 ml. of absolute ethanol containing 150 ml. of distilled water and 12.0 g. of potassium cyanide. After standing overnight, the white precipitate was washed with 90% ethanol, absolute ethanol, ether, and petroleum ether (30-60°C.). The process was repeated with a second 10.0-g. portion of GM-OAc-PC. The O-methyl glucomannan N-methyl-phenylcarbamate (GM-OCH_3-MPC) was finally obtained as a fine white powder, 17.0 g. (77.8%). Anal. calcd. for $C_6H_7O_2(OCH_3)_{0.23}(OCONCH_3Ph)_{2.77}$: OCH_3, 1.33; N, 7.28. Found: OCH_3, 129; N, 7.23.

The infrared spectrum of the GM-OCH_3-MPC was characteristic of a disubstituted urethan, Fig. 4A. The N-H band at 3.05 μ and the amide II band at 6.55 μ were both absent indicating that the phenylcarbamoyl group had been N-methylated. The marked sharpening of the carbonyl band at 5.80 μ indicated the loss of the O-acetyl carbonyl.

The absence of O-acetyl groups in the methylation product was verified in the following manner. A portion of the product (0.489 g.) was dissolved in 8.7 ml. of tetrahydrofuran, and 0.56 ml. of 33% sulfuric acid was added giving a final concentration of 2% sulfuric acid. After standing at room temperature for seven days, ethanol was added and the precipitate was washed and

*Silver oxide was prepared by adding one liter of sodium hydroxide solution (125 g.) to two liters of hot silver nitrate solution (540 g.). The precipitated oxide was washed with boiling water until neutral to litmus. After washing with methanol the oxide was dried in vacuo at 40°C. over phosphorus pentoxide and stored over calcium chloride.

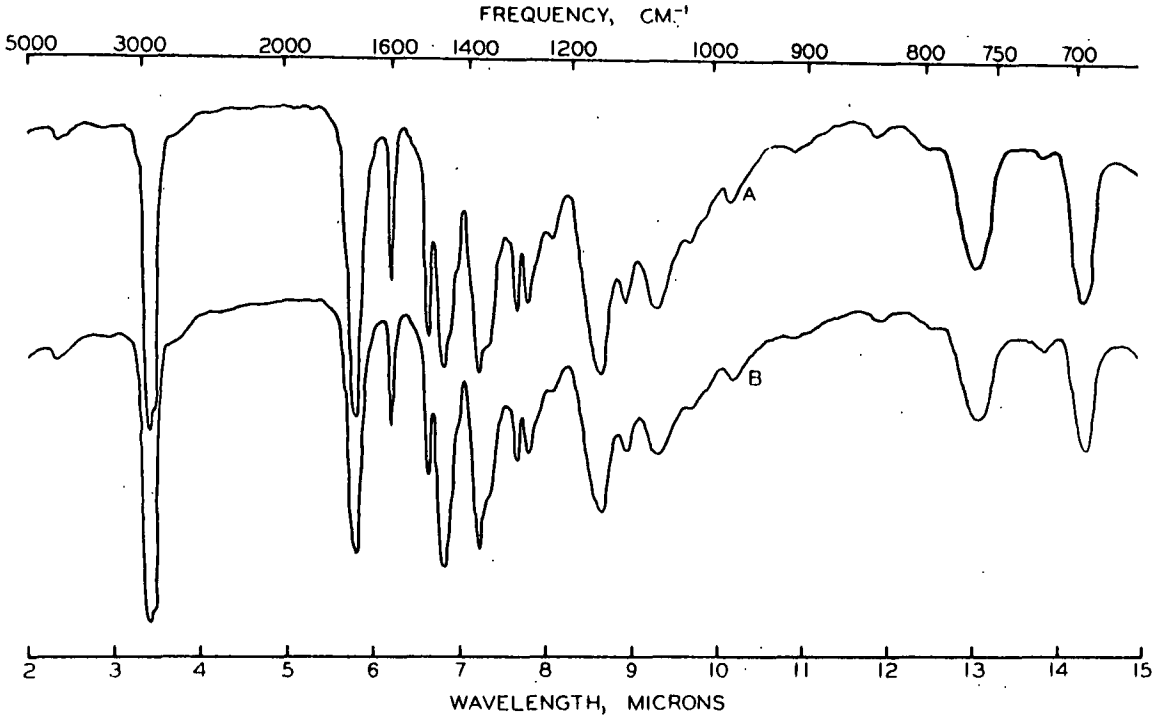


Figure 4. (A) Infrared Spectrum of O-Methyl Glucomannan N-Methyl-Phenylcarbamate. (B) Same Material After Treatment with 2% Sulfuric Acid for Seven Days (Phase-Nujol)

dried in the usual manner. The infrared spectrum of the acid-treated GM-OCH_3-MPC contained no hydroxyl bands at 2.95 μ. The spectra of the GM-OCH_3-MPC before and after the acid treatment were identical as shown in Fig. 4.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF O-METHYL GLUCOMANNAN N-METHYL-PHENYLCARBAMATE

Five grams of dried GM-OCH_3-MPC were dissolved in 200 ml. of dry tetrahydrofuran* (b.p. 65.5-66.5°C.) in a 500-ml. three-necked round-bottom flask equipped with a reflux condenser and a calcium chloride drying tube. Powdered

*Tetrahydrofuran was dried by distilling from potassium hydroxide.

lithium aluminum hydride* (1.5 g.) was added slowly while the solution was stirred magnetically. A thick gel formed in about 20 minutes and was dispersed by refluxing for one hour with continued stirring. After cooling, an additional 0.75 g. of lithium aluminum hydride was added and the mixture stirred continuously at room temperature for a total of 10 hours. The flask was then placed in a room temperature water bath and the excess lithium aluminum hydride destroyed by the dropwise addition of distilled water over a period of a few hours. The mixture was acidified to pH 6 with 5% phosphoric acid and allowed to stand overnight. The tetrahydrofuran was decanted and the residue extracted with tetrahydrofuran on a sintered-glass filter. The tetrahydrofuran extract gave a negative Molisch test and was discarded. The residue was then extracted with cold water (2800 ml.) until a negative Molisch test was obtained. The residue still gave a positive Molisch test so it was further extracted with hot water in a Soxhlet extractor for four days. This time only a faint Molisch test was obtained for the extract, while the residue still gave a positive test. The cold and hot water extracts were processed separately. They were concentrated, dialyzed for one week against distilled water, and freeze-dried. Qualitative chromatography in Solvent C (Appendix II) and methoxyl analysis indicated that the cold and hot water extracts were identical, so they were combined. An additional 5.9 g. of GM-OCH_3-MPC were reduced in a similar manner and all the extracts combined to give 2.41 g. (69.8%) of O-methyl glucomannan (GM-OCH_3). Anal. calcd. for $C_6H_7O_2(OCH_3)_{0.23}(OH)_{2.77}$: OCH_3, 4.32. Found: OCH_3, 1.28 (see discussion on p. 36).

*Lithium aluminum hydride was obtained from Metal Hydrides Inc., Beverly, Mass.

The infrared spectrum of the GM-OCH_3 showed that the reduction was complete, Fig. 5. The aromatic bands at 6.24, 6.66, 13.1, and 14.35 μ were all absent, indicating that the N-methylphenylcarbamoyl blocking groups had been removed.

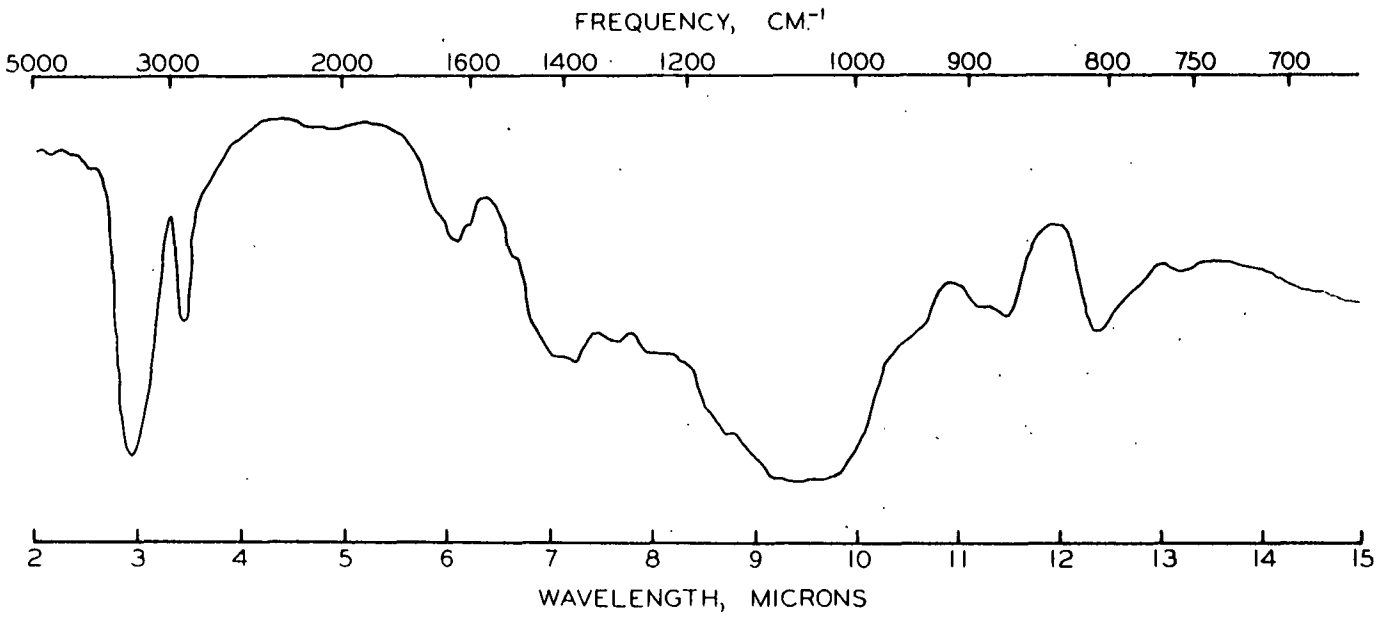


Figure 5. Infrared Spectrum of O-Methyl Glucomannan (Phase-KBr)

The over-all yield of the O-methyl glucomannan based on the natively acetylated glucomannan was 34%.

CHARACTERIZATION OF O-METHYL GLUCOMANNAN

Hydrolysis of O-Methyl Glucomannan

To about 0.5 g. of GM-OCH_3 in a 50-ml. round-bottom flask were slowly added 5.0 ml. of 72% sulfuric acid, and the mixture held at room temperature for one hour with occasional stirring. By this time the GM-OCH_3 had dissolved to give a dark brown solution. Then 25 ml. of distilled water were added to give a final concentration of 12% sulfuric acid. The flask was maintained at 90°C. for

five hours while the contents were stirred magnetically. The hydrolyzate was cooled, and neutralized to pH 5.5 with saturated barium hydroxide. The mixture was centrifuged and the salts were washed twice with 50% aqueous ethanol. The supernatant liquid was concentrated *in vacuo* at 45°C. Additional quantities of GM-OCH_3 were treated in a similar manner until 1.8 g. had been hydrolyzed.

Chromatographic examination of the GM-OCH_3 hydrolyzate in Solvents B, C, and D using *p*-anisidine hydrochloride spray revealed the presence of a component with a mobility and color reaction (yellow-brown) comparable to a monomethyl hexose. There were no indications of higher methylated sugars when the hydrolyzate was chromatographed together with 2,3,4,6-tetra-*O*-methyl-D-glucose in Solvent C.

A 50-mg. portion of the GM-OCH_3 was quantitatively analyzed for sugars (60) and the following composition was obtained: galactose 5.7%, glucose 17.5%, mannose 65.4%, arabinose 1.2%, xylose 6.0%, and monomethyl component 3.4%. The monomethyl component was determined with a calibration curve prepared for 4-*O*-methyl-D-glucose*.

Isolation of Methylated Sugars

The monomethyl fraction was isolated from the GM-OCH_3 hydrolyzate by preparative paper chromatography using Whatman No. 17 filter paper with a wick of Whatman No. 1 filter paper (Appendix II). The hydrolyzate was streaked in equal portions along seven inches of three nine-inch wide sheets. After conditioning overnight, the chromatograms were developed for 35 hours in Solvent C. One-inch guide strips were cut from the sides of the chromatograms and the

*Obtained through the courtesy of R. J. Ross.

monomethyl fraction located with p-anisidine hydrochloride. The corresponding sections were removed from the unsprayed portions of the chromatograms, eluted with distilled water, and concentrated in vacuo at 45°C. (55 mg.). The monomethyl component accounted for 42% of the methylated hexoses theoretically available from the GM-OCH_3 (based on the methoxyl content of 1.28%).

Six milligrams of the monomethyl fraction were demethylated with 48% hydrobromic acid (Appendix II). Chromatographic examination of the demethylation products in Solvent A showed that both glucose and mannose were parent sugars, indicating the presence of at least two methylated sugars in the monomethyl fraction.

The monomethyl fraction was separated into two components by developing in Solvent A for 24 hours. Both components gave a positive reaction when sprayed with 2,3,5-triphenyltetrazolium chloride reagent. Only reducing sugars having a free hydroxyl at carbon 2 can give a positive reaction with this spray reagent (75).

A better separation of the two components in the monomethyl fraction was obtained by developing in Solvent E for 50 hours. The slower moving component (I) had a mobility ($R_{Glucose}$ 3.0) and color reaction to p-anisidine hydrochloride (yellow-brown) that was identical to that of authentic 3-O-methyl-D-glucose. The faster moving major component (II) ($R_{Glucose}$ 4.7) also gave a yellow-brown color with p-anisidine hydrochloride. Authentic 2-O-methyl-D-mannose moved faster than component II ($R_{Glucose}$ 5.5) and gave a maroon color with p-anisidine hydrochloride.

The monomethyl fraction was resolved into components I and II by preparative paper chromatography in Solvent E. The monomethyl fraction (49 mg.) was

streaked across five inches of a six-inch wide sheet of Whatman 3MM filter paper. After conditioning overnight and developing in Solvent E for 72 hours, one-half-inch guide strips were removed and the components located with silver nitrate reagent. The components were eluted from the paper with distilled water and the sodium removed with ion exchange resin IR-120(H)*. The borate was removed as methyl borate by distillation with methanol.

Ratio of Methylated Sugars

After chromatographic separation in Solvent E, the ratio of components I and II was determined colorimetrically according to the method of Saeman, et al. (60). The monomethyl fraction was spotted on Whatman No. 1 filter paper and developed in Solvent E for 72 hours. Guide strips were also spotted and used to locate the components using silver nitrate reagent. The components were eluted from the chromatogram and placed in separate 3-ml. volumetric flasks. Then 0.375 ml. of Somogyi reagent was added and the stoppered flasks heated in a boiling water bath for 20 minutes. After cooling, 0.375 ml. of Nelson reagent was added and the volume brought to 3.0 ml. with distilled water. The absorbance of each solution was read at 520 mμ against a blank prepared in the same manner using distilled water. The average ratio of absorbances (determined in quadruplicate) for component II to component I was 2.5.

Identification of Methylated Sugars

The anilide of component I was prepared according to the method of Irvine and Hogg (76). Component I was placed in a 3-ml. test tube and dried with an air stream. Then 50 μl. of aniline and a few drops of methanol were added.

*A product of Rohm & Haas Co., Philadelphia, Pa.

The mixture was kept at 50°C. for two hours and again dried with an air stream. Ether was added and the mixture allowed to stand overnight. The product was washed with ether and recrystallized from methanol. The anilide had a m.p.[1] of 158-159°C. and a mixed m.p. of 157-159°C. with an authentic sample of N-phenyl-3-O-methyl-D-glucopyranosylamine (m.p. 158-159°C.)[2]. The anilide yield was about 50% based on the amount of component I theoretically available from the monomethyl fraction. The infrared spectrum of the anilide was identical with that of the authentic sample of N-phenyl-3-O-methyl-D-glucopyranosylamine, Fig. 6.

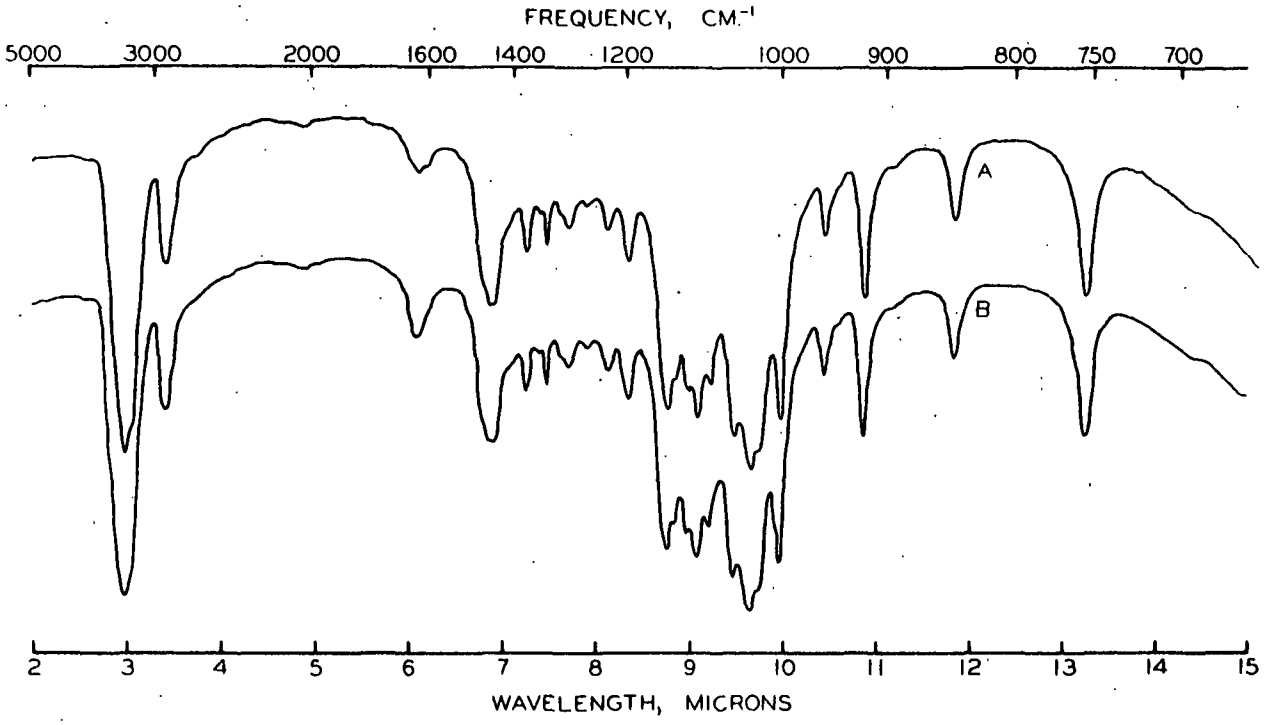


Figure 6. Infrared Spectra of Anilides Prepared from (A) 3-O-Methyl-D-Glucose and (B) Component I (Phase-KBr)

[1] All melting points are corrected.

[2] This derivative was prepared from authentic 3-O-methyl-D-glucose (m.p. 157-159°C.) according to the method of Irvine and Hogg (76).

Five milligrams of component II were demethylated with 48% hydrobromic acid. Examination of the demethylation products in Solvent A showed that mannose was the parent sugar.

The phenylosazone of component II was prepared according to the method of Hassid and McCready (77). Component II was placed in a 3-ml. test tube and diluted to 1 ml. with distilled water. In a separate 3-ml. test tube, 300 mg. of sodium acetate and 200 mg. of phenylhydrazine hydrochloride were dissolved in 1 ml. of warm water. The contents of the tubes were mixed and kept at 90°C. for one hour. The phenylosazone crystallized overnight and was washed by centrifuging, first with three 2-ml. portions of 10% acetic acid and then with three 2-ml. portions of distilled water. The phenylosazone had a m.p. of 161-163°C. and a mixed m.p. of 164-166°C. with authentic 3-O-methyl-D-arabino-hexose phenylosazone (m.p. 165-166°C.)*. The phenylosazone yield was about 35% based on the amount of component II theoretically available from the monomethyl fraction. The infrared spectrum of the phenylosazone was identical with that of the authentic sample of 3-O-methyl-D-arabinohexose phenylosazone, Fig. 7.

SUMMARY AND DISCUSSION OF REACTION SEQUENCE EMPLOYED FOR LOCATION OF O-ACETYL GROUPS

The reaction sequence utilized for locating the O-acetyl groups in the natively acetylated glucomannan is shown in Fig. 8. The natively acetylated glucomannan (I) was swollen in dimethylformamide and treated with an excess of phenylisocyanate to convert the free hydroxyl groups to phenylcarbamoyl groups (II). Nitrogen and O-acetyl analyses and infrared spectra showed that no acetyl groups were lost and that substitution was essentially complete.

*This derivative was prepared from authentic 3-O-methyl-D-glucose (m.p. 157-159°C.) according to the method of Hassid and McCready (77).

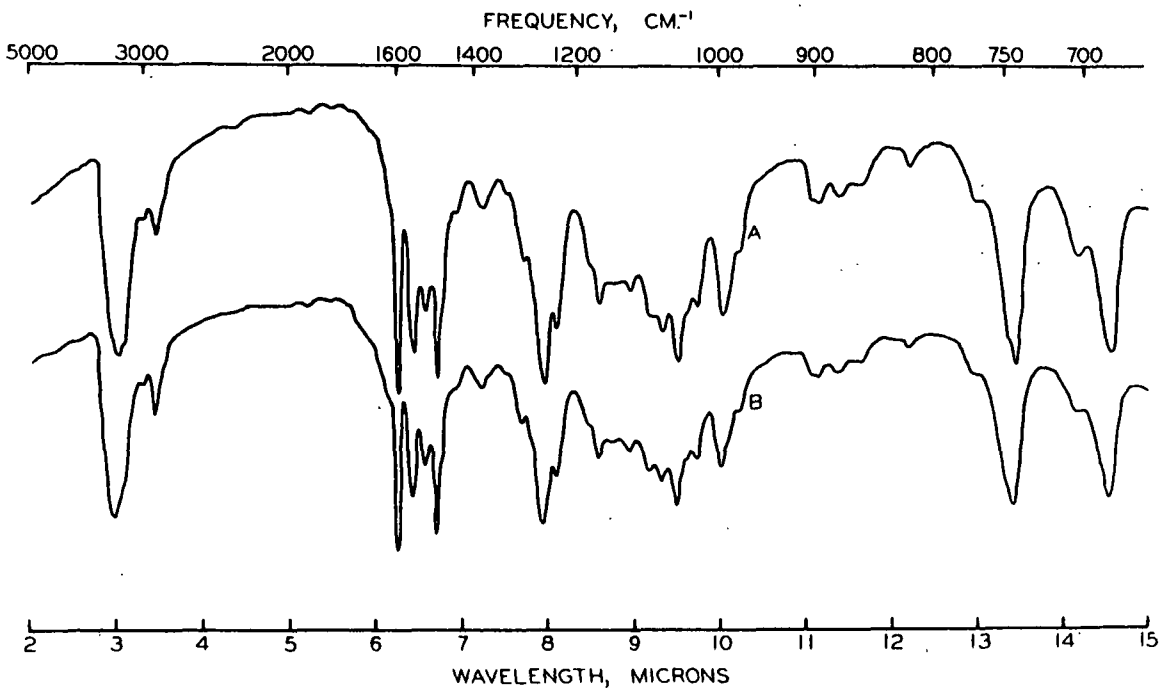
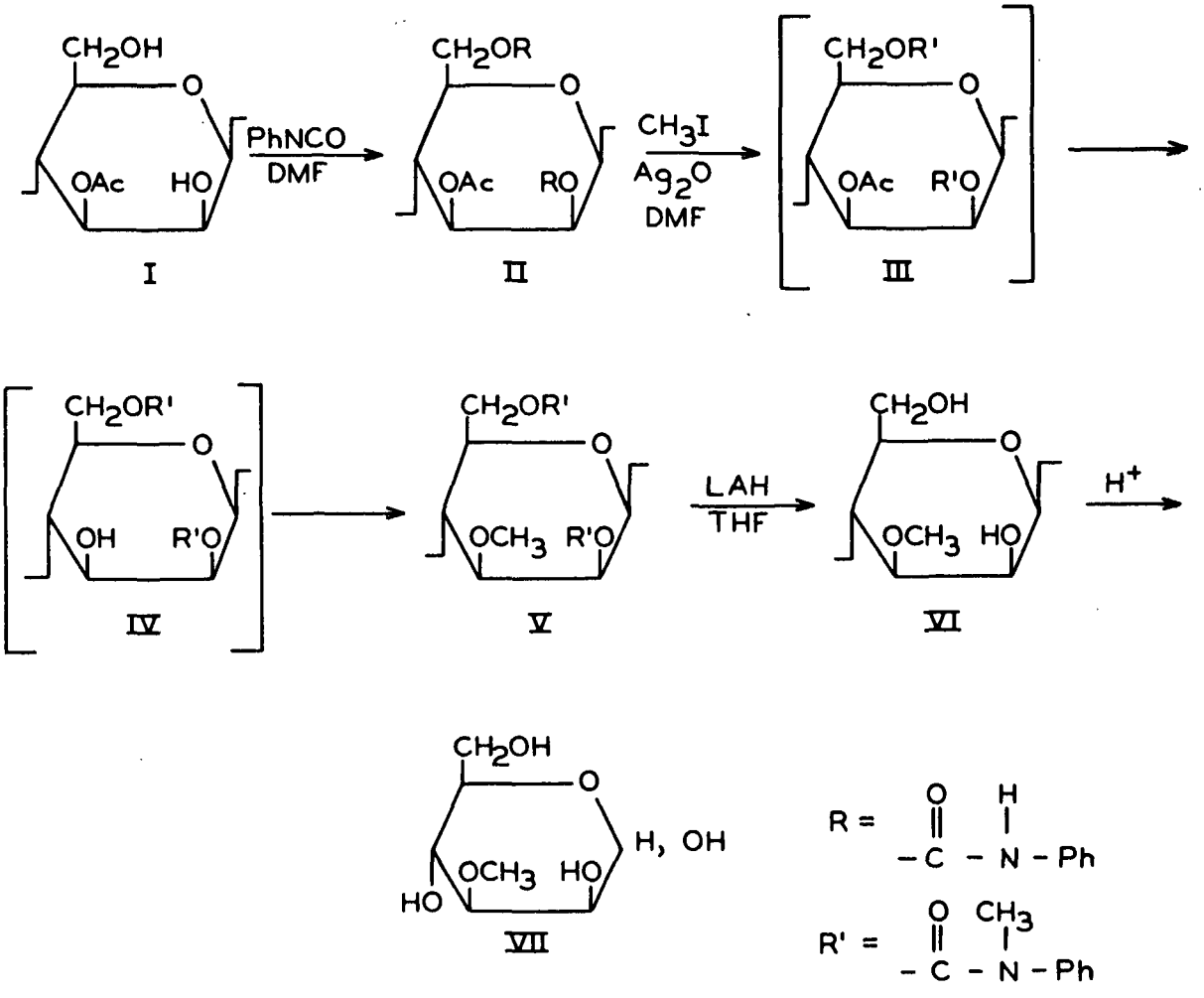


Figure 7. Infrared Spectra of Phenylosazones Prepared from (A) 3-O-Methyl-D-Glucose and (B) Component II (Phase-KBr)

At this point a mild acid hydrolysis would normally be employed for the removal of the O-acetyl groups and the resulting deacetylated polymer methylated in a separate step (20, 66). In this work, however, it was found that both the deacetylation and subsequent methylation could be carried out in a single step. When the O-acetyl glucomannan phenylcarbamate (II) was treated by the Kuhn procedure (78) (the polymer was dissolved in dimethylformamide and treated with methyl iodide and silver oxide) the O-acetyl groups were replaced by O-methyl groups and the phenylcarbamoyl groups were N-methylated all in one step, to give the desired O-methyl glucomannan N-methyl-phenylcarbamate (V). The initial reaction in this conversion of II to V was probably N-methylation of the phenylcarbamoyl groups to give N-methyl-phenylcarbamoyl groups (III), followed by saponification of the O-acetyl groups (IV), and finally, methylation of the resulting free hydroxyl groups (V).



(I) Natively Acetylated Glucomannan

(II) O-Acetyl Glucomannan Phenylcarbamate

(V) O-Methyl Glucomannan N-Methyl-phenylcarbamate

(VI) O-Methyl Glucomannan

DMF = Dimethylformamide

LAH = Lithium Aluminum Hydride

THF = Tetrahydrofuran

Figure 8. Reaction Sequence Employed for Locating the O-Acetyl Groups in the Natively Acetylated Paraña Pine Glucomannan

Deacetylation and methylation of the resulting free hydroxyls during the Kuhn methylation (78) of partially acetylated carbohydrates was observed only recently by Bouveng, et al. (19) and Garegg (66), and is usually considered an objectionable side reaction resulting from the alkalinity of the silver oxide. In this work, however, the reaction was quite useful since it allowed three desirable reactions to be carried out without the isolation of intermediate products.

The absence of O-acetyl groups in the O-methyl glucomannan N-methyl-phenylcarbamate was verified by treating it with 2% sulfuric acid in moist tetrahydrofuran for seven days. Under these conditions any O-acetyl groups would be removed in two days (73). However, no hydroxyl bands could be detected in the infrared spectra of V either before or after the acid treatment, which indicated that the replacement of the O-acetyl groups by O-methyl groups was complete. The composition of V was also confirmed by nitrogen and methoxyl analyses.

The N-methyl-phenylcarbamoyl blocking groups were removed by reduction with lithium aluminum hydride in tetrahydrofuran. The infrared spectrum of the resulting O-methyl glucomannan (VI) indicated that the removal of the blocking groups was complete. The distribution of methoxyl groups in the O-methyl glucomannan should have corresponded to the distribution of the O-acetyl groups in the acetylated glucomannan (I). Unfortunately, methoxyl groups were lost during the preparation of the O-methyl glucomannan. The O-acetyl content of the acetylated glucomannan corresponded to one O-acetyl group for every 5 hexose units, while the methoxyl content of the O-methyl glucomannan corresponded to one methoxyl group for every 15 hexose units.

The loss of methoxyl groups during the lithium aluminum hydride reduction of methylated mono- and polysaccharides has been observed by other investigators.

Chanda, et al. (79) treated methyl 2,3-di-O-methyl-D-mannopyranoside with lithium aluminum hydride, and after hydrolysis of the reduction products, they found chromatographic evidence of a monomethyl mannose in addition to 2,3-di-O-methyl-D-mannose. However, they did not determine whether the monomethyl fraction contained one or two isomers. Garegg (66) obtained traces of demethylation products when the partially acetylated benzyl 4-O-methyl-β-D-xylopyranosides were subjected to carbanilation, deacetylation, methylation, lithium aluminum hydride reduction, and hydrolysis. When Bouveng (20) treated an O-acetyl-4-O-methylglucuronoxylan by the same series of reactions, he also observed some loss of methoxyl groups.

Croon, et al. (80) studied the demethylation of certain xylose and glucose derivatives under various conditions of acid hydrolysis. Comparable amounts of 2- and 3-O-methyl-D-xylose were formed during the demethylation of 2,3-di-O-methyl-D-xylose. Similarly, comparable amounts of 2- and 3-O-methyl-D-glucose and 2,3-, 2,6-, and 3,6-di-O-methyl-D-glucose were formed from 2,3-di-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-glucose, respectively. These results indicated that the substances studied did not show any selective demethylation, and that the methoxyl groups in different positions seemed to show comparable reactivities toward demethylation under the test conditions. The authors felt that any highly selective demethylation was improbable.

The investigations of Chanda, et al. (79), Garegg (66), and Bouveng (20) do not indicate that the demethylation of carbohydrates during lithium aluminum hydride reduction is a selective reaction. Random demethylation of xylose and glucose derivatives during acid hydrolysis has already been established by Croon, et al. (80). However, these studies were concerned with the demethylation of a single sugar and not mixtures of sugars (glucose and mannose) as in the present case. In general, however, these investigations indicate that the loss of

methoxyl groups during the preparation of the O-methyl glucomannan could be assumed to have occurred at random, i.e., methoxyls could be lost from any position of either mannose or glucose with equal probability.

The demethylation that occurred during the preparation of the O-methyl-glucomannan made it apparent that it would not be possible to obtain a direct quantitative measure of the original O-acetyl distribution by characterization of the O-methyl glucomannan. However, the location of the remaining O-methyl groups could still be determined.

The first step in locating the positions of the O-methyl groups was total hydrolysis of the O-methyl glucomannan as indicated in Fig. 9. Qualitative chromatography of the hydrolyzate in Solvents B, C, and D indicated that only a monomethyl hexose fraction was present in addition to the expected unmethylated sugars (galactose, glucose, mannose, arabinose, and xylose). There were no indications of higher methylated sugars.

The monomethyl fraction was isolated from the total hydrolyzate by preparative paper chromatography in Solvent C. On demethylation of this fraction, both mannose and glucose were obtained as parent sugars, indicating the presence of at least two components.

The monomethyl fraction was finally resolved into two components by preparative paper chromatography in Solvent E. Component I gave the same anilide as 3-O-methyl-D-glucose. Component II gave mannose on demethylation, and the same phenylosazone as 3-O-methyl-D-glucose, which indicated that component II was 3-O-methyl-D-mannose.

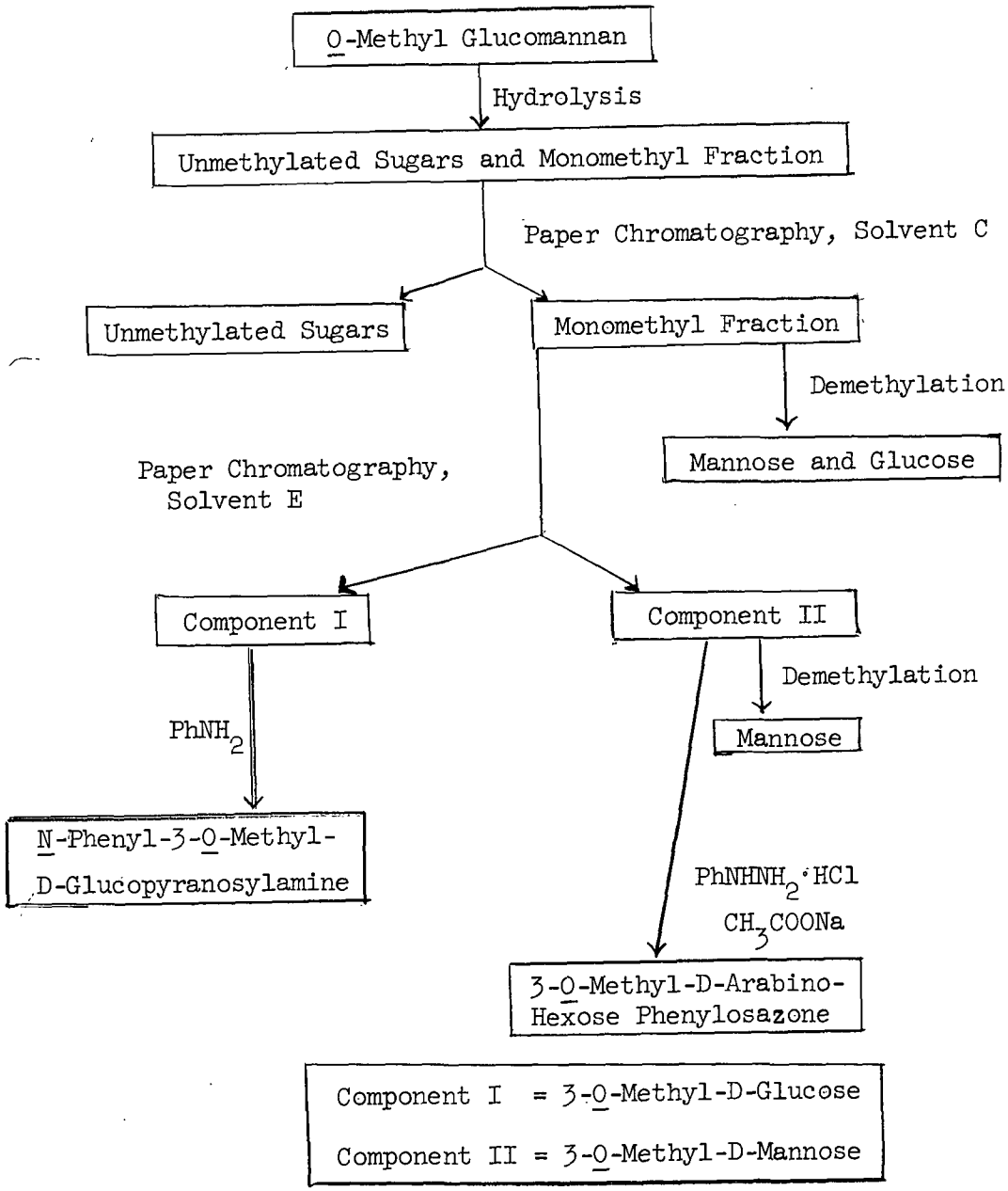


Figure 9. Summary of Isolation and Characterization of Methylated Sugars Obtained from the O-Methyl Glucomannan

A quantitative measure of the O-acetyl distribution was not possible because of the demethylation that occurred during the preparation of the O-methyl glucomannan. However, if one accepts the assumption of random demethylation mentioned earlier, then only the ratio of the methylated sugars is necessary to obtain an indirect measure of the original O-acetyl distribution.

The methylated sugars in the monomethyl fraction were separated chromatographically and the ratio determined colorimetrically. The ratio of component II to component I was 2.5. Thus, in the natively acetylated glucomannan (5.86% O-acetyl), 15.6% of the mannose units and 6.4% of the glucose units carried O-acetyl groups in the 3-position, while none were found in other positions.

CHARACTERIZATION OF NATIVELY ACETYLATED GLUCOMANNAN

Previous studies of natively acetylated glucomannans (24-27) have reported only the carbohydrate and O-acetyl analyses of these polymers. It was felt that additional information should be obtained for the acetylated glucomannan isolated in this work. The acetylated polymer was studied by infrared spectroscopy in addition to determining its intrinsic viscosity in cupriethylenediamine and optical rotation in water and alkali.

INFRARED SPECTRA

The acetylated glucomannan (GM-OAc) and the corresponding deacetylated polymer (GM-D) were first studied by infrared spectroscopy. The GM-D was prepared by dissolving the GM-OAc in 0.1N sodium hydroxide and allowing the solution to stand overnight. After deionizing with IR-120(H) and IR-45(OH), the solution was concentrated and freeze-dried.

A sample of Glazier's (54) alkali-extracted Paraña pine glucomannan (GM-B) was also obtained for infrared examination*. The polymer was originally dried from petroleum ether after solvent exchange and was in the form of a tan powder. To obtain a sample suitable for infrared examination, the polymer was dissolved in 2% sodium hydroxide, and after removal of the sodium with IR-120(H) the solution was concentrated and freeze-dried.

*The sample was obtained from E. E. Dickey, The Institute of Paper Chemistry, Appleton, Wis. The polymer contained galactose, glucose, and mannose in the ratio of 0.08:1:2.6. Additional information may be found in Appendix IV.

The results of the infrared studies are given in Fig. 10. Absorption bands characteristic of softwood glucomannans (81, 82) were observed at 11.45 μ and 12.45 μ for the GM-OAc, GM-D, and GM-B. The spectra of GM-D and GM-B were almost identical.

The acetylated glucomannan also contained three absorption bands characteristic of cellulose acetates, i.e., C=O stretch at 5.75 μ, C-CH_3 deformation at 7.28 μ, and C-O stretch at 8.05 μ (83).

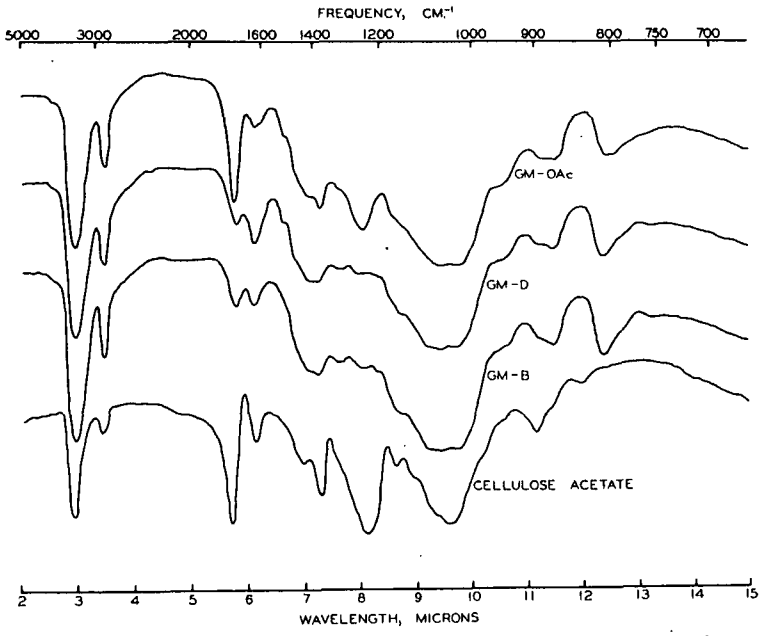


Figure 10. Infrared Spectra of Natively Acetylated Paraña Pine Glucomannan (GM-OAc), Corresponding Deacetylated Glucomannan (GM-D), Glazier's (54) Alkali-Extracted Paraña Pine Glucomannan (GM-B), and Cellulose Acetate (40.5% O-Acetyl) (Phase-KBr)

OPTICAL ROTATION

Optical rotations were obtained for the GM-OAc, GM-D, and GM-B. To obtain the rotation of the GM-D in water the GM-OAc was deacetylated with 0.1N sodium hydroxide, deionized with IR-120(H) and IR-45(OH), and concentrated to an appropriate volume. The exact concentration was determined by removing an aliquot which was first freeze-dried, and then dried at 105°C. to constant weight.

Rotations were obtained with a Zeiss-Winkel polarimeter using sodium light. Polarimeter tubes of either 0.5 or 2.0 decimeters in length were used depending on the clarity of the solutions. The calculated rotations are listed in Table VII.

TABLE VII

OPTICAL ROTATIONS

Sample	Solvent	$[\alpha]_D^{28}$, degrees	Concentration, g./dl.
GM-OAc	Water	-28.7	0.50
GM-D	Water	-24.7	0.73
GM-OAc	10% NaOH	-25.3	1.00
GM-B	10% NaOH	-35.9	0.78

The optical rotations of the acetylated glucomannan in water and alkali were similar to those obtained for alkali-extracted softwood glucomannans ($-30 \pm 10°$) as shown in Table I.

INTRINSIC VISCOSITY

The intrinsic viscosity of the acetylated glucomannan in 1M cupriethylenediamine* was determined at 30.0 ± 0.005°C. using a Cannon Ubbelohde No. 100 viscometer. The solution as well as the solvent used for subsequent dilutions were filtered through a sintered-glass microfilter. A plot of the reduced viscosity versus concentration extrapolated to infinite dilution gave an intrinsic viscosity of 0.35 dl./g. as shown in Fig. 11.

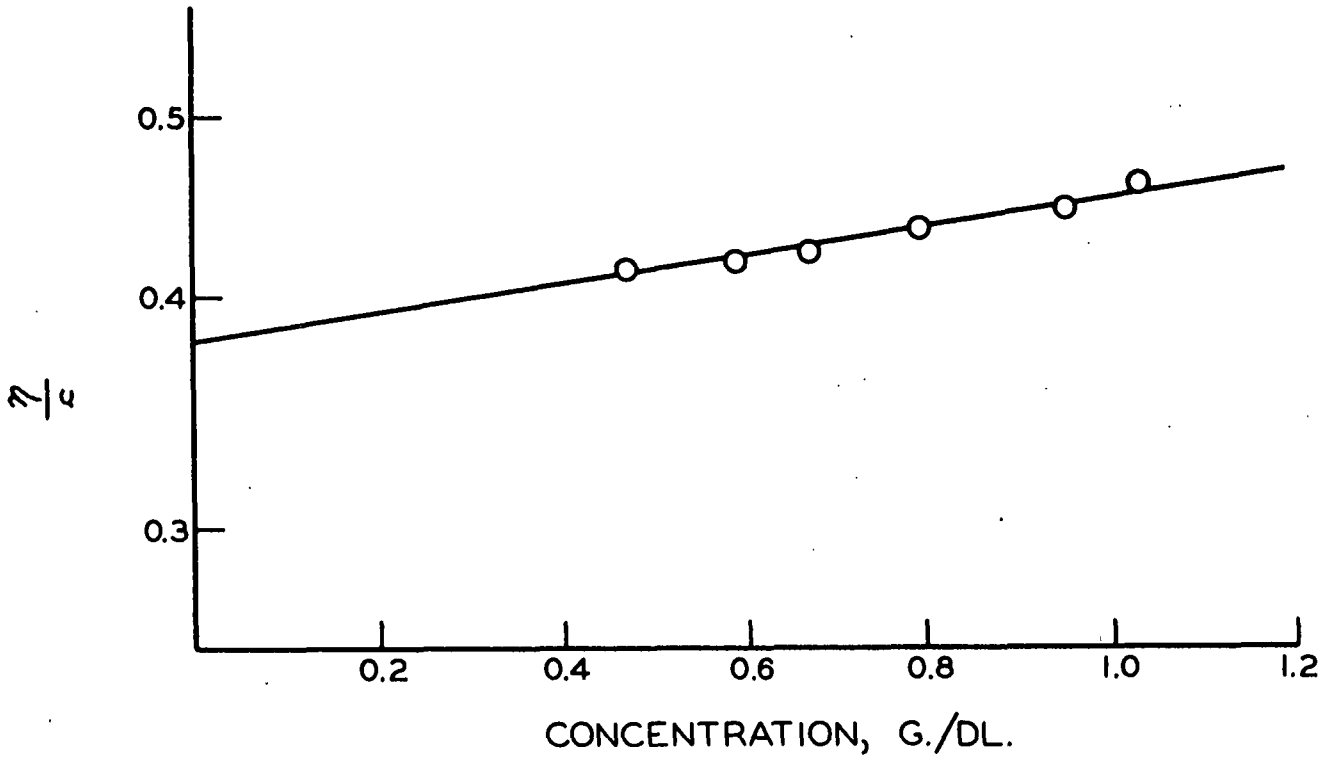


Figure 11. Log of Reduced Viscosity Versus Concentration for Natively Acetylated Paraña Pine Glucomannan in 1M Cupriethylenediamine at 30.0 ± 0.005°C.

*Ecusta Cellulose Solvent manufactured by Olin Mathieson Chemical Co., Pisgah Forest, N. C.

The presence of the O-acetyl groups probably does not have much effect on the cupriethylenediamine intrinsic viscosity of the acetylated glucomannan, since the solvent is sufficiently alkaline to remove these groups. Consequently, the value of 0.38 dl./g. obtained for the acetylated glucomannan is probably quite close to the intrinsic viscosity that would be obtained for the acetylated polymer after removal of the O-acetyl groups.

The value of 0.38 dl./g. compares fairly well with 0.41 dl./g. obtained by Glazier (54) for his alkali-extracted Paraña pine glucomannan. Both of these values are well within the range of intrinsic viscosities reported for other alkali-extracted softwood glucomannans (0.2 to 0.5 dl./g.) as shown in Table I. The significantly high cupriethylenediamine intrinsic viscosity of the acetylated glucomannan obtained in this study may indicate that the method of isolation did not involve appreciable degradation of this polymer.

It would be desirable to obtain a value for the degree of polymerization of the acetylated polymer from its intrinsic viscosity in cupriethylenediamine. Unfortunately, the exact relationship between these two quantities is not known and usually an independent determination of the molecular weight must be made on an organic derivative of the hemicellulose in question. The white spruce glucomannan isolated by Vaughan (46) had an intrinsic viscosity of 0.34 dl./g. while the number average degree of polymerization determined for the nitrate ester was 70. When these values are inserted into the equation $\overline{DP}_n = K[\eta]$ the resulting value K is 206*. Using this value of K and the cupriethylenediamine intrinsic viscosity of 0.38 dl./g. the $\overline{DP}_n$ of the acetylated glucomannan is 78, which is in the same range of values that have been obtained for alkali-extracted glucomannans as shown in Table I.

*This is based on the assumption that a (in the equation $[\eta] = KM^a$) is approximately unity, which may not be as good an assumption with hemicelluloses as with cellulose.

EFFECT OF THE O-ACETYL GROUPS ON THE PHYSICAL PROPERTIES OF THE ACETYLATED GLUCOMANNAN

INTRODUCTION

The influence of the O-acetyl groups on the physical properties of the acetylated glucomannan was studied by polarization microscopy, x-ray diffraction, and electron microscopy. However, before proceeding with a discussion of the results, it may be helpful to define the terms orientation and degree of lateral order as employed in the present discussion.

Orientation will refer to the alignment of individual macromolecules relative to one another and to the surfaces of the sample, with particular reference to the regions of little or no order (84). Lateral order will refer to the degree of regularity of atoms and atomic groups in the direction normal to the chain axis (85). The two extreme cases of lateral order include an amorphous material which has no lateral order and a crystalline material which has a relatively high degree of lateral order.

Orientation will not refer to individual molecules in a crystalline region except in the sense that crystallite orientation determines the orientation of all of its component molecules. It is thus theoretically possible to have a material of relatively low order and parallel molecular orientation, or a highly ordered material with random crystallite orientation. Within limits, the orientation can be changed without appreciably affecting the lateral order, and vice versa.

The ability of a material with relatively low order to maintain varying amounts of orientation has been observed in certain cellulose derivatives. Haas, et al. (86) found that ethyl cellulose films cast on glass gave a diffuse

x-ray pattern indicating the absence of any lateral order. However, examination by polarization microscopy showed that the films were uniaxially oriented and had a high cross-sectional birefringence. After annealing, the films were still unordered, but no longer exhibited birefringence. The annealing had produced a randomly oriented structure. Completely unoriented and unordered films could be produced directly by casting them on mercury surfaces and allowing them to dry without constraint in the plane of the film.

There are three basic factors which can influence the degree of lateral order developed between polymer molecules: (a) Rigidity of Chains. Polymers having relatively inflexible chains crystallize more easily than those with chains of a high degree of internal flexibility. (b) Nature of Groups in Chains. If strongly polar groups or hydrogen bonding systems are regularly distributed along the length of the individual macromolecules, such as the hydroxyl groups in cellulose, an inherent tendency for the formation of well-ordered domains is produced. The closer the groups and the better their lateral fit, the more pronounced is their effect on the crystallinity. (c) Perfection of Fit. Bulky, nonpolar, and irregularly distributed substituents which tend to keep the individual chain molecules apart and prevent the establishment of hydrogen bonding, noticeably decrease the tendency for the development of highly ordered domains (87).

Dispersion or van der Waals forces usually account for most of the attractive energy between molecules. These forces, as well as dipole interactions, are effective only at very short distances, of the order of a few angstroms. Thus, if two polymer molecules fit together very closely, the total attractive energy will be large. If the structures along the chain are uniform (as would be the case with an alkali-extracted glucomannan), the perfection of packing

will be improved and the polymer can develop regions of high lateral order. Thus, cellulose with its relatively stiff chains and the hydrogen-bonding capacity of its frequent hydroxyls is able to develop relatively high degrees of crystallinity. However, if only a small number of hydroxyls are replaced by more bulky groups, then the regularity of packing of adjacent chains will be interrupted, decreasing the extent of intermolecular hydrogen bonding and reducing the degree of lateral order. This phenomenon probably accounts for the observed water solubility of the natively acetylated glucomannan, and of cellulose derivatives at fairly low levels of substitution.

Evaluation of orientation and degree of lateral order are generally possible only in terms of a specific experimental technique. In this work, relative changes in molecular orientation were evaluated by polarization microscopy. Differences in the relative degree of lateral order were evaluated by x-ray diffraction.

EXPERIMENTAL

POLARIZATION MICROSCOPY

Freeze-dried samples of the acetylated glucomannan, (GM-OAc), the corresponding deacetylated polymer (GM-D), and Glazier's (54) alkali-extracted Paraña pine glucomannan (GM-B) were mounted in mineral oil on a one by three-inch glass microscope slide. The slides were viewed with a Zeiss Polarizing Microscope and photographed at a magnification of 140 diameters.

The polarization photomicrographs are presented in Fig. 12-14. As seen in Fig. 12B, the acetylated glucomannan appeared to be isotropic since areas of double refraction (birefringence) were not observed with crossed nicols.

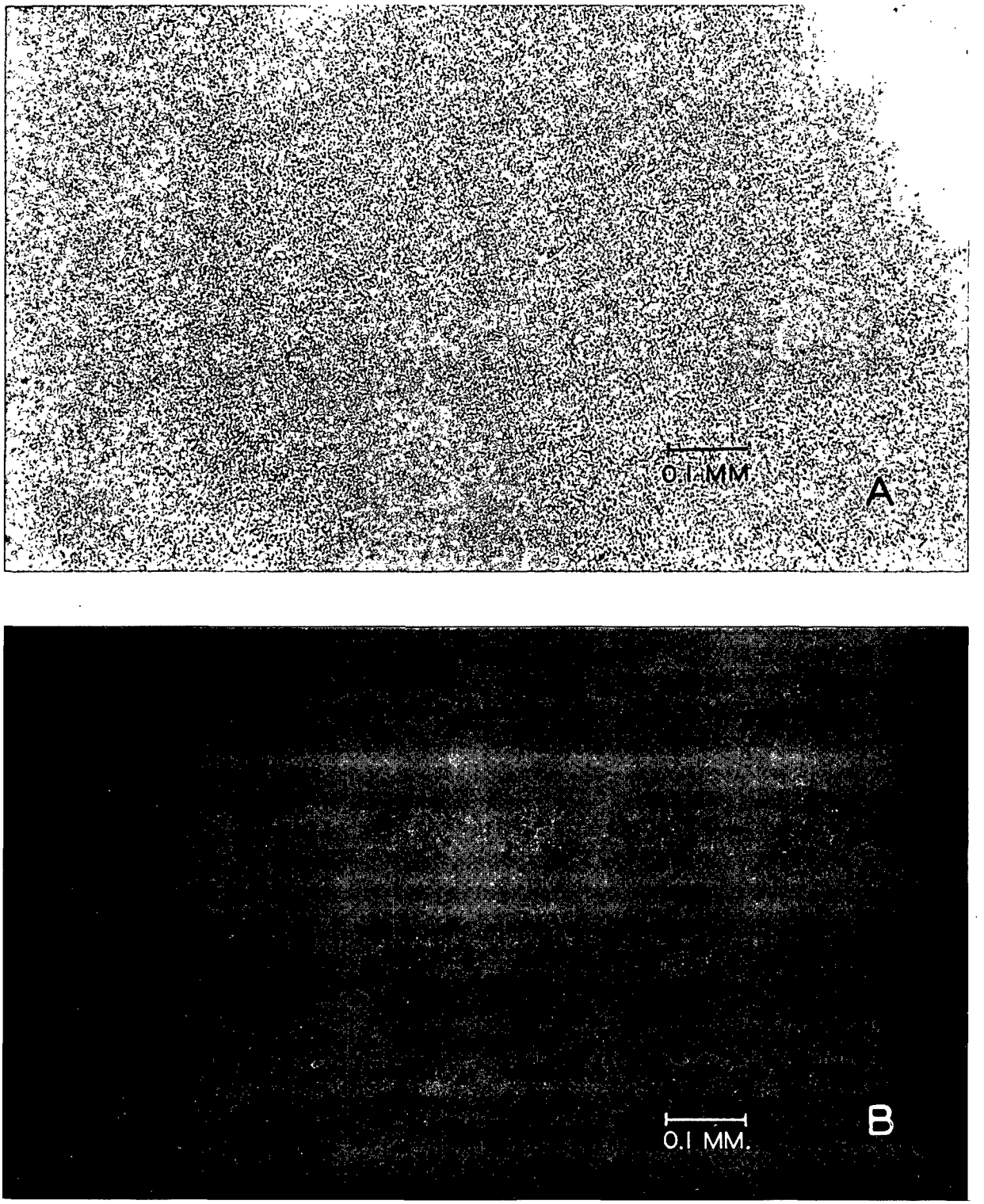


Figure 12. Photomicrograph of Natively Acetylated Paraña Pine Glucomannan (GM-OAc) Taken with Polarized Light. Sample Was Freeze-Dried and Mounted in Mineral Oil, Magnification 140X. (A) Parallel Nicols. (B) Same Field with Crossed Nicols

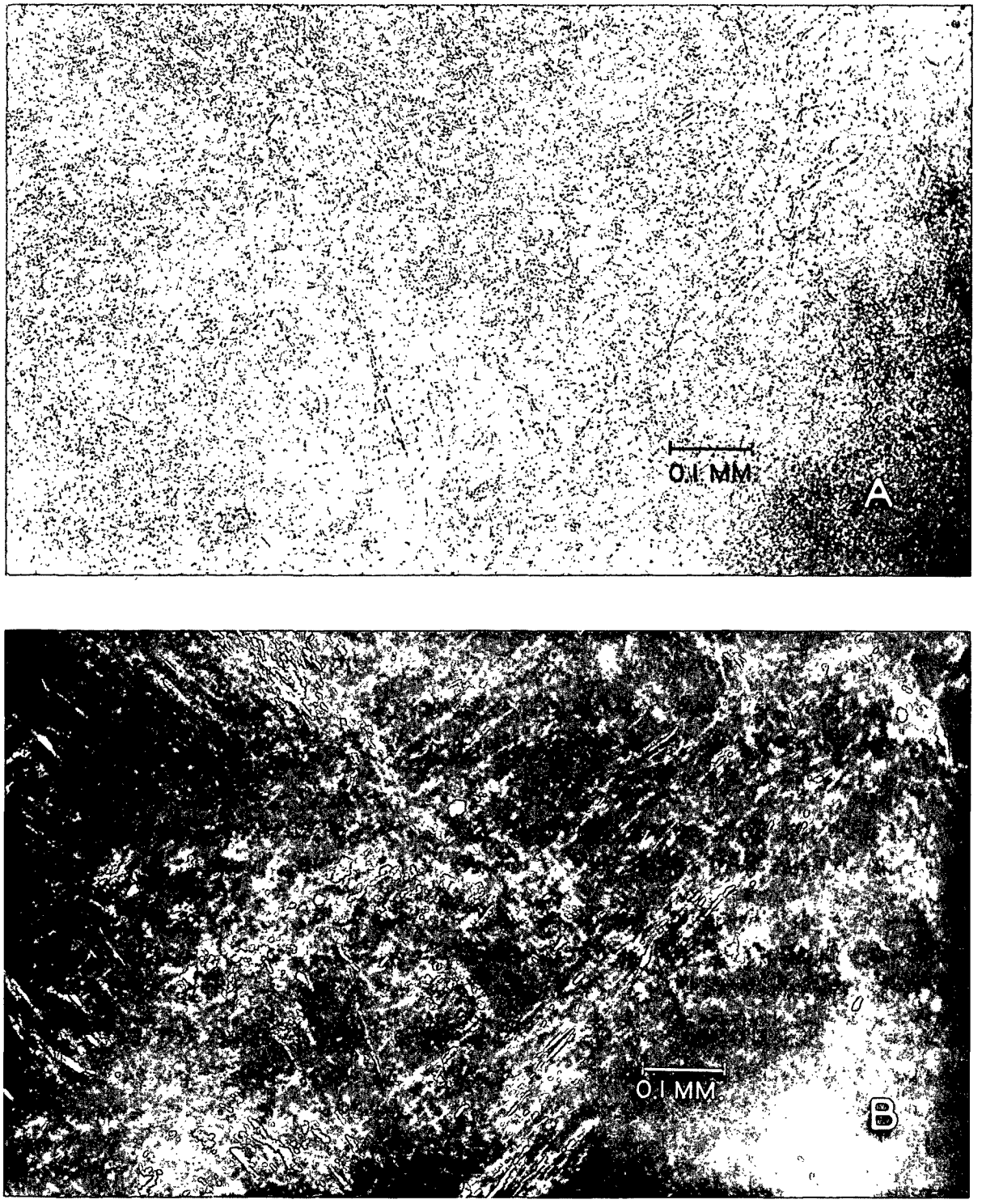


Figure 13. Photomicrograph of Natively Acetylated Paraña Pine Glucomannan After Deacetylation (GM-D) Taken with Polarized Light. Sample Was Freeze-Dried and Mounted in Mineral Oil, Magnification 140X. (A) Parallel Nicols. (B) Same Field with Crossed Nicols

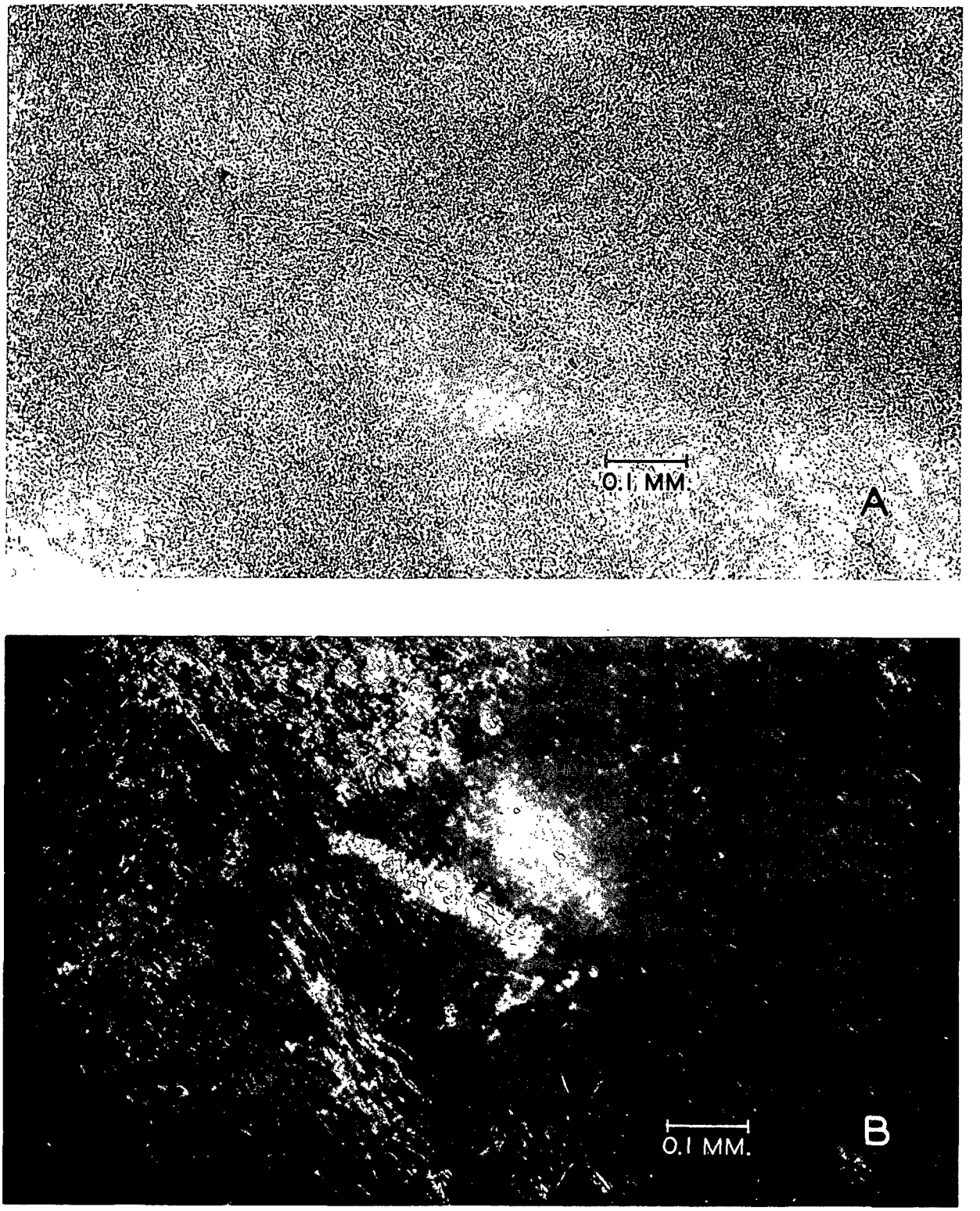


Figure 14. Photomicrograph of Glazier's (54) Alkali-Extracted Paraña Pine Glucomannan (GM-B) Taken with Polarized Light. Sample Was Freeze-Dried and Mounted in Mineral Oil, Magnification 140X. (A) Parallel Nicols (B) Same Field with Crossed Nicols

The deacetylated polymer and Glazier's (54) alkali-extracted glucomannan, on the other hand, both exhibited optical anisotropy since considerable birefrigence was observed with crossed nicols, as shown in Fig. 13B and 14B.

The results of the polarization microscopy study indicate that the acetylated glucomannan maintains a randomly oriented molecular structure as long as the O-acetyl are present, thus accounting for the lack of birefringence in Fig. 12B. Only on removal of the O-acetyl groups does preferred molecular orientation occur, as indicated by the birefringence that was observed for the deacetylated polymer and Glazier's (54) alkali-extracted glucomannan in Fig. 13B and 14B.

Koshijima (88) also observed birefringence in an alkali-extracted glucomannan from Akamatsu wood, which he attributed to enhanced molecular orientation followed by the development of regions with relatively high degrees of lateral order.

X-RAY DIFFRACTION

X-ray diffractograms were obtained for the following materials: (a) GM-OAc which had been dried from ether and had aged one year; (b) GM-D which had been freeze-dried and had not aged more than a few weeks (same sample used for infrared spectroscopy); and (c) Glazier's (54) Paraña pine glucomannan which had been dried from ether and had aged for three years.

A Norelco parafocused diffractometer was employed with a wide-angle goniometer. The tube was run at 35 kv. and 20 ma. Scattering and divergence slits were 1/2°, and the receiving slit was 0.006-inch wide. Nickel-filtered copper x-ray radiation (λ = 1.5418 A.) was used.

The results of the x-ray studies are shown in Fig. 15. The acetylated glucomannan appeared to have maintained an unordered structure even after aging for over a year. This complete lack of order is not usually observed for alkali-extracted glucomannans. These polymers usually give x-ray diffractograms characteristic of more ordered structures, such as those observed by Morak and Ward (47), Matsuzaki, et al. (89), Koshijima (88), and Lindberg and Meier (41), as shown in Fig. 16 and 17. Lindberg and Meier (41) and Matsuzaki, et al. (89) both found that degradation of their glucomannans by mild acid hydrolysis resulted in even more distinct x-ray patterns, which closely resembled that of ivory nut mannan A.

Vaughan (90, 91) prepared a highly ordered glucomannan by crystallizing the polymer from a hot aqueous solution (70°C.) and drying it from water. The glucomannan crystals were about 2.5 μ in length, exhibited strong birefringence, and gave an x-ray diffractogram characteristic of a well-ordered structure as shown in Fig. 18.

The deacetylated polymer GM-D appeared to have developed only a slight amount of order relative to the acetylated polymer GM-OAc. Only the alkali-extracted glucomannan obtained by Glazier (54), which had aged about three years, gave a diffractogram indicative of a slightly ordered structure.

The x-ray diffraction studies indicate that the acetylated glucomannan maintains an unordered structure as long as the O-acetyl groups are present. This is in agreement with the polarization microscopy studies, since an ordered structure cannot be formed without prior molecular orientation. However, once the O-acetyl groups are removed and preferred molecular orientation occurs, the tendency to form an ordered structure through intermolecular

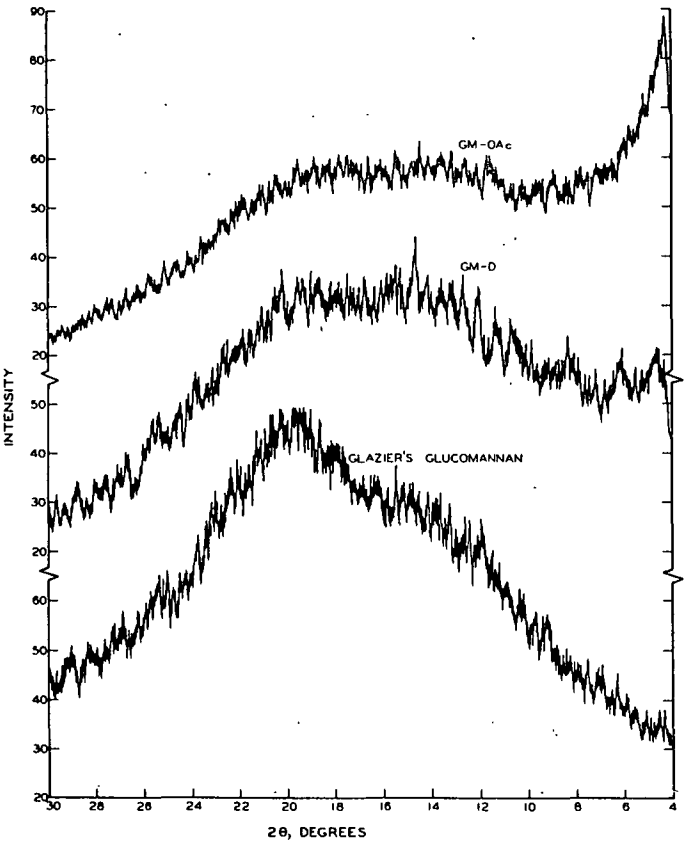


Figure 15. X-Ray Diffractograms of Isolated Glucomannans

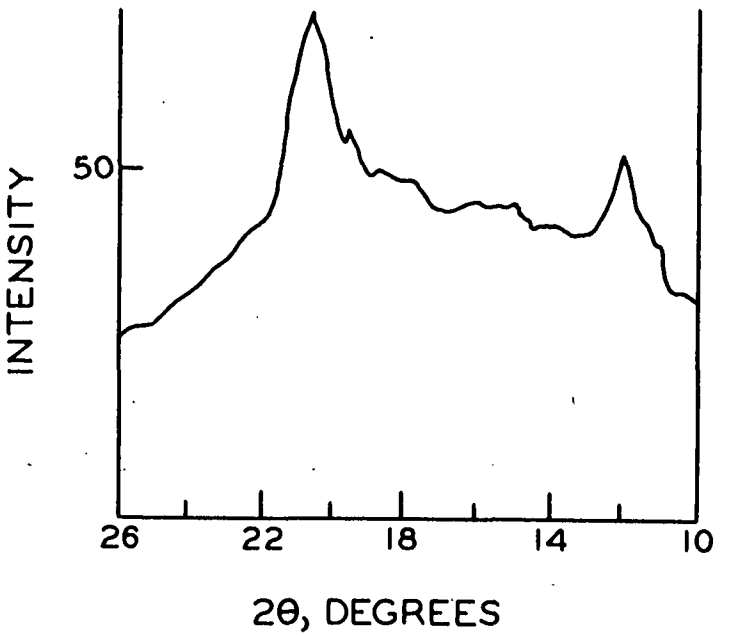


Figure 16. X-Ray Diffractogram of a Glucomannan Isolated from a Black Spruce Holocellulose (47)

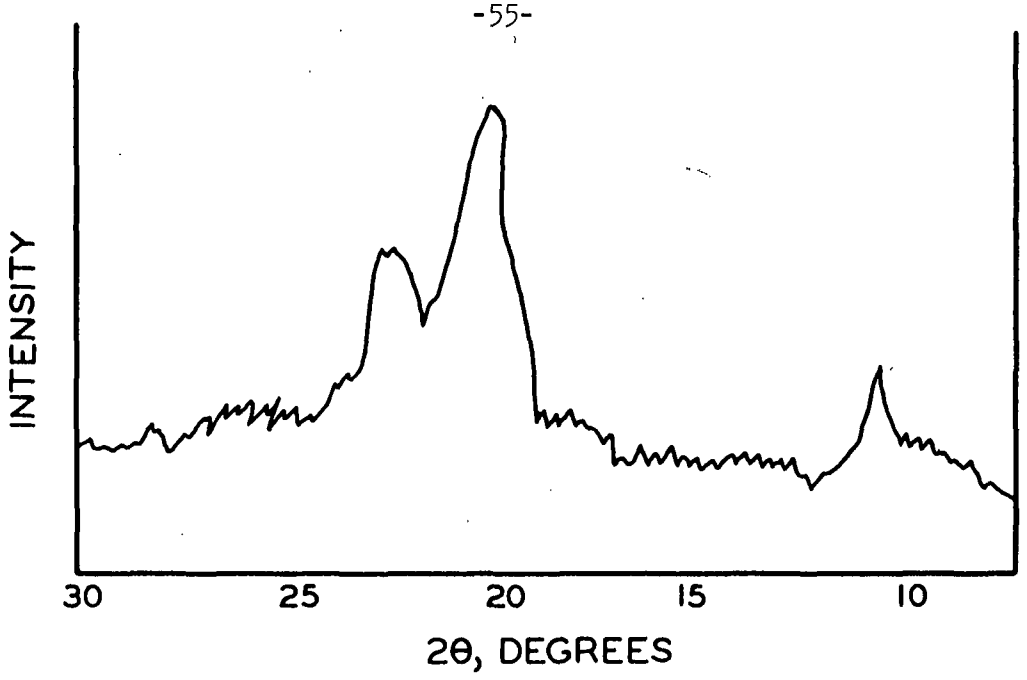


Figure 17. X-Ray Diffractogram of a Glucomannan Isolated from an Akamatsu Holocellulose, Fraction (b) (88)

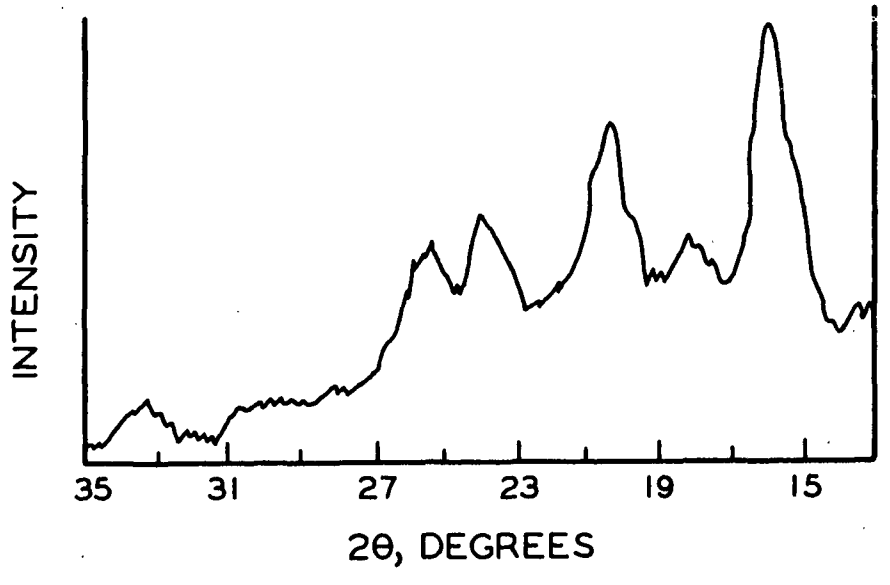


Figure 18. X-Ray Diffractogram of a Crystalline Glucomannan Isolated from a Slash Pine Holocellulose (91)

hydrogen bonding is enhanced. The development of lateral order does not seem to occur immediately after deacetylation but appears to be time dependent, as can be seen from the diffractograms of the acetylated and corresponding deacetylated polymers in Fig. 15. This gradual development of lateral order following deacetylation would account for the ordered structures other investigators (47, 88, 89) have found for their alkali-extracted glucomannans by x-ray diffraction. Apparently, the process may also be accelerated by hydrolytic treatments as was shown by Lindberg and Meier (41), Matsuzaki, et al. (89), and Vaughan (91). In any case, it appears that the prerequisite for the formation of an ordered structure is the removal of the O-acetyl groups.

ELECTRON MICROSCOPY

The aqueous solutions of the GM-OAc and GM-D employed for optical rotations were also used for the electron microscopy studies. The solution to be examined was placed on a collodion grid and the water removed under vacuum. The deposited hemicellulose was shadowed with palladium at an angle of 30° and then viewed and photographed using an RCA electron microscope, Type EMU-3F, operated at 50 kv.

The electronmicrographs are presented in Fig. 19 and 20. The acetylated glucomannan was composed of nearly spherical individual particles with a fairly narrow distribution of particle sizes. The corresponding deacetylated polymer, on the other hand, was composed of irregularly shaped particles which appeared to be quite agglomerated. The deacetylated polymer had its own characteristic structure, and did not appear to be formed by the aggregation of discrete particles such as those observed for the acetylated polymer. It seems that by restricting the glucomannan molecules to randomly oriented configurations, the O-acetyl groups have caused the acetylated polymer to form discrete regularly

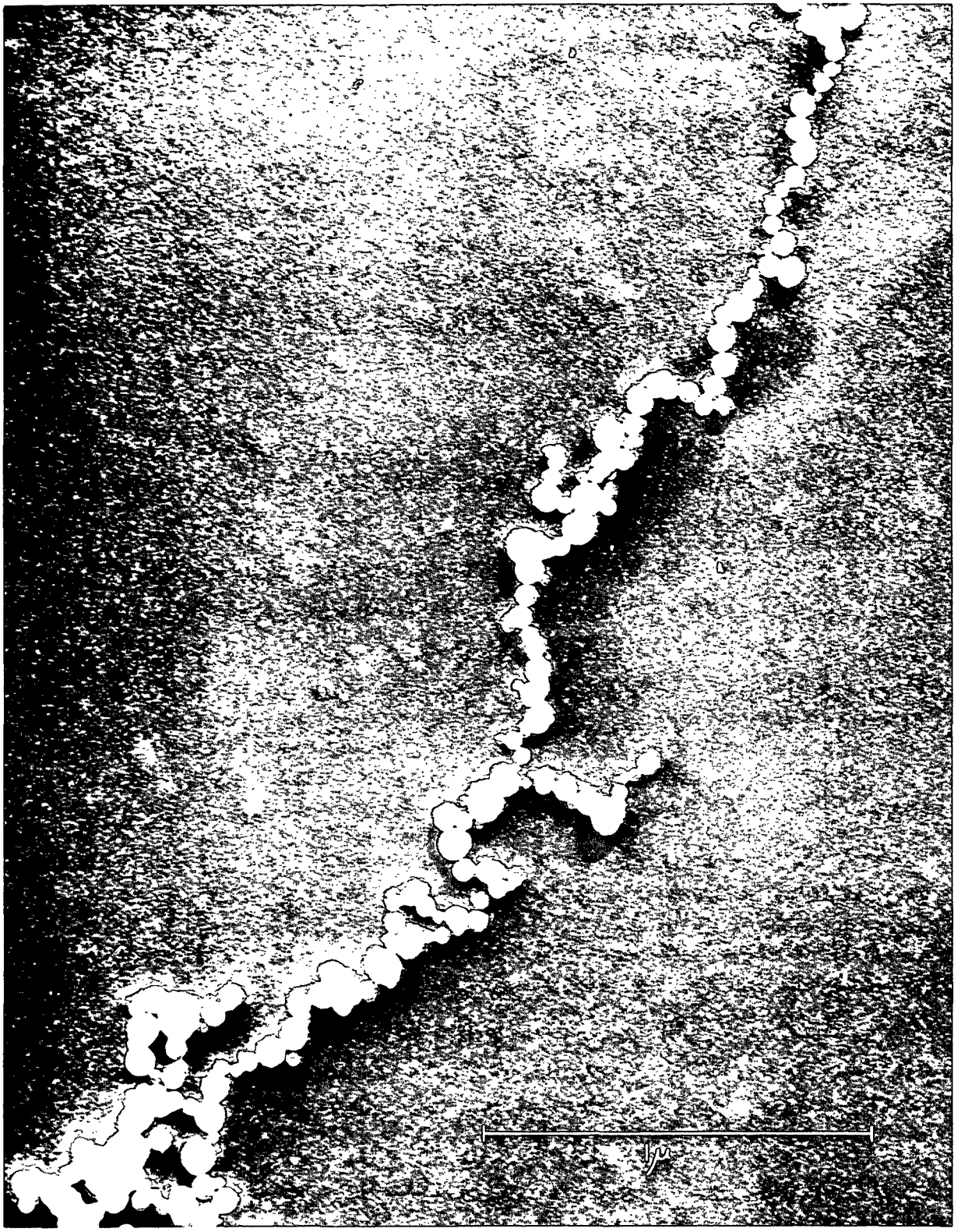


Figure 19. Electronmicrograph of Natively Acetylated Paraña Pine Glucomannan (GM-OAc), Palladium Shadowed at 30°, Magnification 67,000X

Figure 20. Electronmicrograph of Natively Acetylated Paraña Pine Glucomannan After Deacetylation (GM-D), Palladium Shadowed at 30°, Magnification 67,000X

shaped particles when deposited from an aqueous solution on a collodion grid. Once the O-acetyl groups are removed and a preferred molecular orientation is developed, the opportunity for intermolecular hydrogen bonding is enhanced, and may indeed be responsible for the apparently agglomerated particles observed for the deacetylated polymer. The fact that the acetylated polymer appeared to be deposited in the form of beads linked together on a string was interesting, but no explanation for this condition is yet available.

SOLUBILITY

The natively acetylated glucomannan isolated in this work remained water soluble for over a year after its isolation, and showed no decrease in solubility. This type of solubility behavior is not characteristic of alkali-extracted glucomannans. Many workers (47, 51, 92-95) found that their alkali-extracted glucomannans were soluble in water and dilute alkali immediately after isolation. However, following an undefined aging period, the water solubility disappeared and higher concentrations of alkali and alkaline borate had to be employed to dissolve these polymers. This behavior may reflect the gradual development of lateral order following the loss of the O-acetyl groups.

DISCUSSION OF RESULTS

Although sugar and O-acetyl contents have been reported for other natively acetylated glucomannans (24-27), to the author's knowledge, the present work contains the first report of intrinsic viscosity, optical rotation, and infrared data for an acetylated glucomannan. These analyses of the natively acetylated Paraña pine glucomannan indicate that this polymer is similar to the deacetylated glucomannan obtained on alkaline extraction of this wood, and is probably representative of the glucomannan portion of the wood. The acetylated polymer also appears to be representative of other alkali-extracted softwood glucomannans.

To locate the O-acetyl groups in the acetylated glucomannan, the polymer was subjected to a reaction sequence in which the free hydroxyls were converted to phenylcarbamoyl groups by treatment with phenylisocyanate in dimethylformamide. Subsequent methylation by the Kuhn procedure (78) replaced the O-acetyl groups with methoxyl groups and N-methylated the phenylcarbamoyl groups. Removal of the N-methylphenylcarbamoyl groups by reduction with lithium aluminum hydride should have afforded an O-methyl glucomannan with a distribution of methoxyl groups corresponding to the distribution of O-acetyl groups in the acetylated glucomannan. Regrettably, methoxyl groups were lost during the preparation of the O-methyl glucomannan. No suitable alternative method for removing the N-methylphenylcarbamoyl blocking groups could be found. These groups can be removed by alkali (70), but the accompanying degradation of the polysaccharide would undoubtedly have interfered with the subsequent isolation and characterization of the methylated sugars in the O-methyl glucomannan.

A review of the literature showed that the demethylation of carbohydrates is probably not a selective reaction (p. 36). Consequently, it was assumed

that the demethylation that occurred during the preparation of the O-methyl glucomannan was completely random, i.e., methoxyls could be lost from any position of either mannose or glucose with equal probability.

The demethylation made it apparent that it would not be possible to obtain a direct quantitative measure of the original O-acetyl distribution. However, the location of the remaining O-methyl groups could still be determined. The O-methyl glucomannan was hydrolyzed, and the methylated sugars were resolved by preparative paper chromatography and suitably characterized. The only methylated sugars found in the O-methyl glucomannan were 3-O-methyl-D-glucose and 3-O-methyl-D-mannose.

If one accepts the assumption of random demethylation occurring during the preparation of the O-methyl glucomannan, then only the ratio of the methylated sugars is necessary to obtain an indirect measure of the original O-acetyl distribution in the acetylated glucomannan. The ratio of 3-O-methyl-D-mannose to 3-O-methyl-D-glucose was 2.5. Thus, in the natively acetylated glucomannan (5.86% O-acetyl), 15.6% of the mannose units and 6.4% of the glucose units carried O-acetyl groups in the 3-position.

The results of this work are consistent with the periodate oxidation studies of Meier (24) and Koshijima (25) which indicated that the O-acetyl groups in their respective acetylated glucomannans were primarily attached to the mannose units. However, they were not able to detect the small proportion of glucose units that may also have carried O-acetyl groups. This of course assumes that all natively acetylated glucomannans have the same distribution of O-acetyl groups, an assumption that will no doubt be clarified in the future.

Without attempting to elucidate the actual mechanism of biological acetylation in wood, it is of interest to compare the results of acetylation studies of model compounds with the results obtained in this and other studies of natively acetylated polysaccharides.

Jeanloz and Jeanloz (96) demonstrated that the type of acylating agent employed in partial acylation studies exerted a considerable influence on the distribution of the acyl groups in the product. When methyl 4,6-benzylidene-β-D-glucopyranoside was partially acetylated with acetic anhydride in pyridine, the product was substituted mainly in the 3-position. On the other hand, the use of acetyl chloride favored the 2-position.

Partial acetylation studies on benzyl 4-O-methyl-β-D-xylopyranoside by Garegg (66) showed that with acetic anhydride and sodium acetate, the hydroxyl at the 2-position was more reactive, while the hydroxyl at the 3-position was more reactive with acetic anhydride and perchloric acid. Other systems, such as acetic anhydride and pyridine containing pyridine hydrochloride gave distributions of the O-acetyl groups showing lesser degrees of preferential reaction than those two extremes.

Hermans, et al. (97) studied the reactivity of vicinal diols to acetylation, and determined the absolute rates of acetylation for cis- and trans-1,2-cyclohexane diols. They found that the equatorial hydroxyls reacted 3.7 times as fast as the axial hydroxyls. In terms of wood polysaccharides, the results indicated that the intrinsic reactivity of the hydroxyls at the 2- and 3-positions of cellulose and xylan would be greater than for a mannan under the given acetylating conditions. Unfortunately, comparative data on the homogeneous acetylation of polysaccharides is not available.

With reference to acetylation in nature, the temperate hardwoods have a higher O-acetyl content (3 to 5%) than the softwoods (about 1%). The hardwood O-acetyl groups are associated with the 4-O-methylglucuronoxylan (9 to 17% O-acetyl), while in the softwoods they are associated with the glucomannan (5 to 8% O-acetyl). For an O-acetyl-4-O-methylglucuronoxylan from white birch (13.2% O-acetyl) Bouveng (20) found that 11.8% of the xylose units carried O-acetyl groups in the 2-position, 24.0% in the 3-position, and 6.1% in both the 2- and 3-position. The exclusive acetylation of the 3-position in the glucomannan was surprising when compared to the distribution of O-acetyl groups observed for the natively acetylated white birch xylan. However, the O-acetyl content of the xylan was about twice that of the glucomannan.

The precise biological role of the O-acetyl groups has yet to be established. These groups may function differently in hardwoods and softwoods since in each case they are associated with a different hemicellulose. There is sufficient evidence (24, 27) to indicate that all the softwood O-acetyl groups are associated with the glucomannan just as the hardwood O-acetyl groups appear to be associated with the 4-O-methylglucuronoxylan (98). However, the question of the distribution of these O-acetyl groups among the polymer molecules must also be considered, i.e., it is not known whether all the polymer molecules carry O-acetyl groups. Perhaps this information could be obtained from a fractionation study of these natively acetylated hemicelluloses by means of Sephadex resins (99).

The O-acetyl groups were found to have a marked effect on the physical properties of the natively acetylated glucomannan. The results of polarization microscopy, x-ray diffraction, and electron microscopy studies indicate that the glucomannan is probably amorphous in its native state and remains in

this condition even after isolation as long as the O-acetyl groups remain intact. Apparently, the O-acetyl groups in the 3-position of the mannose and glucose units are capable of restricting the glucomannan molecules to randomly oriented configurations, due in part to the bulkiness of the O-acetyl groups relative to the hydroxyl groups. The initial effect of removing these O-acetyl groups is believed to be an increase in intermolecular orientation followed by gradual time dependent development of lateral order through intermolecular hydrogen bonding. In support of this theory it was observed that the natively acetylated glucomannan isolated in this work remained water soluble, appeared isotropic to polarized light, and gave a diffuse x-ray diffractogram even after aging for over a year. Alkali-extracted (deacetylated) glucomannans, on the other hand, become insoluble in alkali, show birefringence under polarized light, and give x-ray diffractograms indicative of ordered structures on aging after isolation.

The ability of the O-acetyl groups to prevent orientation and development of lateral order in natively acetylated glucomannans should have a significant effect on the role of this hemicellulose during pulping. Although the O-acetyl groups are saponified during alkaline pulping, they have been found to be relatively stable during acid sulfite pulping (23, 100). Both Annergren, et al. (27, 101, 102) and Wennerås (103) found that when a sprucewood acid sulfite cook was preceded by a neutral or slightly alkaline bisulfite precook, the glucomannan in the pulp was more resistant to acid hydrolysis than in the absence of the precook. The stabilized portion of the glucomannan was appreciable, and amounted to about 4% of the weight of the wood (65% of the original hemicellulose). The degree of polymerization of the stabilized glucomannan was about 30, indicating an average of two ruptures per molecule based on a degree of polymerization of 100 for the undegraded glucomannan.

The observed glucomannan stabilization may be accounted for in the following manner. In the alkaline precook (pH 8 or 9) the O-acetyl groups are removed and the glucomannan molecules become highly oriented with respect to each other and the cellulose. On acidification some chain cleavage occurs, followed by sorption of the deacetylated glucomannan molecules on the cellulose microfibrils. Intercrystallization of glucomannan chains through intermolecular hydrogen bonding probably also occurs. The net result is an increase in the degree of lateral order for the glucomannan portion of the pulp with concomitant increase in resistance of the glucomannan to acid hydrolysis. Annergren, et al. (102) showed that the glucomannan could be sorbed on the cellulose during bisulfite-acid sulfite pulping, but the present work demonstrates more clearly how the removal of the O-acetyl groups increases the resistance of the softwood glucomannan to hydrolysis during this pulping process.

ACKNOWLEDGMENTS

The author wishes to acknowledge the guidance of his thesis advisory committee, N. S. Thompson (Chairman), L. E. Wise, and E. E. Dickey. They provided invaluable advice and encouragement during the course of this work. The author also appreciates the many constructive suggestions provided by Mr. H. Swenson during the preparation of this thesis manuscript.

Appreciation is also extended to the personnel at The Institute of Paper Chemistry who assisted in certain aspects of the experimental program: J. Hultman for x-ray diffractograms; J. Hankey for photomicrographs taken with the polarizing microscope; L. Sell for infrared spectra; and Miss O. Smith for electronmicrographs.

The Paraña pinewood used in this investigation was kindly supplied by Mr. F. L. Brown of Lutcher S.A., Sao Paulo, Brazil.

Authentic 3-O-methyl-D-glucose was obtained from A. Kirkland of Washington University. A sample of 2-O-methyl-D-mannose was kindly supplied by Dr. G. O. Aspinall of The University of Edinburgh.

Cellulose acetate (CAD 1459) was provided by Dr. L. B. Genung, Eastman Kodak Co., Rochester, New York.

Finally, I should like to thank my wife Mrs. Sandra Katz for her patience and understanding while this work was in progress, and for her co-operation in the typing of this manuscript.

LITERATURE CITED

1. Hägglund, E., Lindberg, B., and McPherson, J., Acta Chem. Scand. 10:1160-4 (1956).

2. Aronovsky, S. I., and Gortner, R. A., Ind. Eng. Chem. 27:451-4(1935); C.A. 29:3830.

3. Klingstedt, F. W., Finnish Paper and Timber J. 20:140-5(1938); C.A. 32:3606[8].

4. Timell, T. E., Svensk Papperstid. 60:762-6(1957).

5. Whistler, R. L., and Jeanes, A., Ind. Eng. Chem. Anal. Ed. 15:317-18(1943).

6. Pringsheim, H., and Magnus, H., Z. Physiol. Chem. 105:179-85(1919); C.A. 14:1040.

7. Pringsheim, H., and Magnus, H., Z. Angew. Chem. 33 I:56(1920); C.A. 14:2013.

8. Klason, P., Svensk Papperstid. 35:717(1932).

9. Hägglund, E., and Sandelin, O., Svensk Kem. Tid. 46:83-7(1934); C.A. 28: 6303[5].

10. Schmidt, E., Tang, Y. C., and Jandelbauer, W., Cellulosechemie 12:201-12 (1931); C.A. 26:1772.

11. Mitchell, R. L., Rogers, S. C., and Ritter, G. J., Ind. Eng. Chem. 40: 1528-9(1948).

12. Bird, C. D., and Ritter, G. J., J. Am. Chem. Soc. 59:802-3(1937).

13. Kurth, E. F., and Ritter, G. J., J. Am. Chem. Soc. 56:2720-3(1934).

14. Ritter, G. J., and Kurth, E. F., Ind. Eng. Chem. 25:1250-3(1933).

15. Klauditz, W., Holzforschung 11:47-55(1957); C.A. 51:12485h.

16. Mitchell, R. L., and Ritter, G. J., J. Am. Chem. Soc. 62:1958-9(1940).

17. Stewart, C. M., Austral. J. Chem. 6:425-31(1953).

18. Stewart, C. M., and Foster, D H., Austral. J. Chem. 6:431-8(1953).

19. Bouveng, H. O., Garegg, P. J., and Lindberg, B., Acta Chem. Scand. 14: 742-8(1960).

20. Bouveng, H. O., Acta Chem. Scand. 15:96-100(1961).

21. Timell, T. E., J. Am. Chem. Soc. 82:5211-5(1960).

22. Bowering, W. D. S., Marchessault, R. H., and Timell, T. E., Svensk Papperstid. 64:191-4(1961).

23. Öhrn, O. E., and Croon, I., Svensk Papperstid. 63:601-5(1960).

24. Meier, H., Acta Chem. Scand. 15:1381-5(1961).

25. Koshijima, T., J. Japan. Wood Res. Soc. 6:194-8(1960); A.B.I.P.C. 31:3784.

26. Sharkov, V. I., Kuĭbina, N. I., and Solov'eva, Yu. P., Zh. Prik. Khim. 33:2571-5(1960); C.A. 55:8852b.

27. Annergren, G. E., Croon, I., Enstrom, B. F., and Rydholm, S. A., Svensk Papperstid. 64:386-93(1961).

28. Fay, P., Glaudemans, C. P. J., and Marchessault, R. H., Abstracts of Papers Presented at American Chemical Society Meeting, Los Angeles, California, April 1-4, 1963, p. 12D; Chem. Eng. News 41, no. 15:79-80(1963).

29. Hamilton, J. K., Kircher, H. W., and Thompson, N. S., J. Am. Chem. Soc. 78:2508-14(1956).

30. Hamilton, J. K., and Kircher, H. W., J. Am. Chem. Soc. 80:4703-9(1958).

31. Hamilton, J. K., and Thompson, N. S., Pulp Paper Mag. Can. 59, no. 10: 233-41(1958).

32. Thompson, N. S., and Kaustinen, O. Unpublished work. Appleton, Wis., The Institute of Paper Chemistry, 1961, 1962.

33. Timell, T. E., Tappi 45:799-802(1962).

34. Croon, I., Lindberg, B., and Meier, H., Acta Chem. Scand. 13:1299-1304 (1959).

35. Bishop, C. T., and Cooper, F. D., Can. J. Chem. 38:793-804(1960).

36. Gyaw, M. O., and Timell, T. E., Can. J. Chem. 38:1957-66(1960).

37. Ball, D. H., Jones, J. K. N., Nicholson, W. H., and Painter, T. J., Tappi 39:438-43(1956).

38. Jones, J. K. N., and Painter, T. J., J. Chem. Soc. 1957:669-73.

39. Jones, J. K. N., and Painter, T. J., J. Chem. Soc. 1959:573-80.

40. Croon, I., and Lindberg, B., Acta Chem. Scand. 12:453-8(1958).

41. Lindberg, B., and Meier, H., Svensk Papperstid. 60:785-90(1957).

42. Meier, H., Acta Chem. Scand. 14:749-56(1960).

43. Immergut, B., and Rånby, B. G., Svensk Papperstid. 60:573-81(1957).

44. Timell, T. E., and Tyminski, A., Tappi 40:519-22(1957).

45. Tyminski, A., and Timell, T. E., J. Am. Chem. Soc. 82:2823-7(1960).

46. Vaughan, J. The oxidation of a spruce glucomannan with lead tetra-acetate. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1963. 65 p.

47. Morak, A. J., and Ward, K., Jr., Tappi 33:12-22(1961).

48. Mills, A. R., and Timell, T. E., Can. J. Chem. 41:1389-95(1963).

49. Aspinall, G. O., Laidlaw, R. A., and Rashbrook, R. B., J. Chem. Soc. 1957:4444-8.

50. Merler, E., and Wise, L. E., Tappi 41:80-6(1958).

51. Hamilton, J. K., and Partlow, E. V., J. Am. Chem. Soc. 80:4880-5(1958).

52. Mian, A. J., and Timell, T. E., Svensk Papperstid. 63:884-8(1960).

53. Schwarz, E. C. A., and Timell, T. E., Can. J. Chem. 41:1381-8(1963).

54. Glazier, R. H. Unpublished work. Appleton, Wis., The Institute of Paper Chemistry, 1960.

55. Bouveng, H., and Lindberg, B., Methods in structural polysaccharide chemistry. Advan. Carbohydrate Chem. 5:53-89(1960).

56. Hirst, E. L., Pure Appl. Chem. 5:56-66(1962).

57. Aspinall, G. O. Structural chemistry of the hemicelluloses. Advan. Carbohydrate Chem. 14:429-68(1959).

58. TAPPI Standard T 13 m-54.

59. TAPPI Standard T 625 m-48.

60. Saeman, J. F., Moore, W. E., Mitchell, R. L., and Millett, M. A., Tappi 37:336-43(1954).

61. Wise, L. E., Murphy, M., and D'Addieco, A. A., Paper Trade J. 122, no. 2:35-43(1946).

62. Meier, H., Acta Chem. Scand. 12:1911-18(1958).

63. Meier, H., Pure Appl. Chem. 5:37-52(1962).

64. Howsmon, J. A., and Marchessault, R. H., J. Appl. Polymer Sci. 1:313-22 (1959).

65. Timell, T. E., Tappi 44:88-96(1961).

66. Garegg, P. J., Acta Chem. Scand. 16:1849-57(1962).

67. Pacsu, E. Carbohydrate orthoesters. Advan. Carbohydrate Chem. 1:77-127 (1945).

68. Sugihara, J. M. Relative reactivities of hydroxyl groups in carbohydrates. Advan. Carbohydrate Chem. 8:1-44(1953).

69. Hearon, W. M., Hiatt, G. D., and Fordyce, C. R., J. Am. Chem. Soc. 65: 829-33(1943).

70. Hearon, W. M., Hiatt, G. D., and Fordyce, C. R., J. Am. Chem. Soc. 65: 833-6(1943).

71. Wolff, I. A., and Rist, C. E., J. Am. Chem. Soc. 70:2779-82(1948).

72. Hearon, W. M., and Lubsitz, J. L., J. Am. Chem. Soc. 70:296-7(1948).

73. Bouveng, H. O., Acta Chem. Scand. 15:87-95(1961).

74. Randall, H. M., Fowler, R. G., Fuson, N., and Dangl, J. R. Infrared determination of organic structures. New York, N. Y., D. Van Nostrand, 1949. 239 p.

75. Avigad, G., Zelikson, R., and Hestrin, S., Biochem. J. 80:57-61(1961).

76. Irvine, J. C., and Hogg, J. P., J. Chem. Soc. 1914:1386-96.

77. Hassid, W. Z., and McCready, R. M., Anal. Chem. 14:683-6(1942).

78. Kuhn, R., Trischmann, H., and Low, I., Angew. Chem. 67:32(1955); C.A. 50:16812i.

79. Chanda, S. K., Hirst, E. L., Percival, E. G. V., and Ross, A. G., J. Chem. Soc. 1952:1833-7.

80. Croon, I., Herrstrom, G., Kull, G., and Lindberg, B., Acta Chem. Scand. 14:1338-42(1960).

81. Marchessault, R. H., Pure Appl. Chem. 5:107-30(1962).

82. Liang, C. Y., Bassett, K. H., McGinnes, E. A., and Marchessault, R. H., Tappi 43:1017-24(1960).

83. Higgins, H. G., Stewart, C. M., and Harrington, K. J., J. Polymer Sci. 51:59-84(1961).

84. Howsmon, J. A., and Sisson, W. A. Submicroscopic structure. In Ott, Spurlin, and Grafflin's Cellulose and cellulose derivatives. Chapter IV. Section B. New York, Interscience, 1954.

85. International Union of Pure and Applied Chemistry Report on Nomenclature in The Field of Macromolecules. J. Polymer Sci. 8:257-77(1952).

86. Haas, H. C., Farney, L., and Valle, C., J. Colloid Sci. 7:584-99(1952).

87. Mark, H. Interaction and arrangement of cellulose chains. In Ott, Spurlin, and Grafflin's Cellulose and cellulose derivatives. Chapter IV. Section A. New York, Interscience, 1954.

88. Koshijima, T., Agr. Biol. Chem. (Tokyo) 26:98-105(1962); A.B.I.P.C. 33:9757.

89. Matsuzaki, K., Moriya, M., and Sobue, H., Kogyo Kagaku Zasshi 62:865(1959); C.A. 56:1463d.

90. Vaughan, J. M., Wise, L. E., and Borchardt, L., J. Org. Chem. 25:1837-8 (1960).

91. Vaughan, J. M. Unpublished work. The Institute of Paper Chemistry, 1960.

92. Ward, K., Jr., Pure Appl. Chem. 5:77-90(1962).

93. Thompson, N. S. Personal communication, Nov., 1961.

94. Morak, A. J. Personal communication, Nov., 1961.

95. Swenson, H. Personal communication, Nov., 1961.

96. Jeanloz, R. W., and Jeanloz, D. A., J. Am. Chem. Soc. 79:2579-82(1957).

97. Hermans, J. J., Marchessault, R. H., Sarkanen, V., and Wright, R., Paper Presented at the First Dissolving Pulps Conference of the Technical Association of the Pulp and Paper Industry, Montreal, Quebec, June 17-20, 1963.

98. Lindberg, B., Pure Appl. Chem. 5:67-76(1962).

99. Flodin, P. Dextran gels and their application in gel filtration. Uppsala, Sweden, Pharmacia, 1962. 85 p.

100. Annergren, G. E., and Croon, I., Svensk Papperstid. 64:618-23(1961).

101. Annergren, G. E., and Rydholm, S. A., Svensk Papperstid. 62:737-46(1959).

102. Annergren, G. E., and Rydholm, S. A., Svensk Papperstid. 63:591-600(1960).

103. Wennerås, S., Norsk Skogind. 16:118-26(1962).

104. Hough, L., Jones, J. K. N., and Wadman, W. H., J. Chem. Soc. 1950:1702-6.

105. Partridge, S. M., Nature 164:443(1949).

106. Trevelyan, W. E., Proctor, D. P., and Harrison, J. S., Nature 166:444 (1950).

107. Hirst, E. L., Hough, L., and Jones, J. K. N., J. Chem. Soc. 1949:928-33.

108. Shaefer, W. C., and Van Cleve, J. W., Anal. Chem. 28:1290-3(1956).

109. Dutton, G. G. S., and Tamalsa, Y., Can. J. Chem. 40:1146-8(1962).

110. Peat, S., Whelan, W. J., and Edwards, T. E., J. Chem. Soc. 1958:3862-8.

111. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., Anal. Chem. 28:350-6(1956).

APPENDIX I

GLOSSARY OF ABBREVIATIONS

GM-OAc	Natively acetylated Paraña pine glucomannan
GM-D	Glucomannan obtained on deacetylation of GM-OAc
GM-B	An alkali-extracted Paraña pine glucomannan isolated by Glazier (54) (Appendix IV)
GM-OAc-PC	O-Acetyl glucomannan phenylcarbamate
GM-OCH_3-MPC	O-Methyl glucomannan N-methyl-phenylcarbamate
GM-OCH_3	O-Methyl glucomannan
DEAE	Diethylaminoethyl

APPENDIX II

GENERAL METHODS OF ANALYSIS

KLASON LIGNIN

Klason lignin was determined according to Institute Method 13 (TAPPI Standard T 13 m-54). Collection of the precipitated lignin was facilitated by using Whatman No. 40 filter paper instead of the recommended porcelain crucible. Identical results were obtained in either case.

SULFATED ASH

The air-dry sample was placed in a cold muffle furnace and heated until the organic matter was almost completely destroyed. The cooled residue was moistened with 50% sulfuric acid and charred on a hotplate until no additional sulfur trioxide was evolved. Finally, the sample was ignited at 900°C. for 30 minutes, cooled and weighed. (TAPPI Standard T 625 m-48).

NITROGEN

Organic nitrogen was determined by the Hengar technique as described in Institute Method 606.

METHOXYL

The Zeisel procedure described by Institute Method 18 (TAPPI Standard T 2 m-60) was used to determine methoxyl. Anisic acid (20.4% methoxyl) obtained from the National Bureau of Standards was used to standardize the method.

ACETYL

Acetyl was determined by the transesterification method of Whistler and Jeanes (5). The method was standardized with cellulose acetate CAD 1459 (40.5% O-acetyl) obtained from Eastman Kodak Co., Rochester, N. Y.

INFRARED ANALYSIS

A Perkin Elmer Model 21 recording spectrophotometer manufactured by the Perkin Elmer Corp., Norwalk, Conn., equipped with a sodium chloride prism, was employed for obtaining infrared spectra. Samples were prepared as potassium bromide pellets or Nujol mulls.

FREEZE DRYING

Aqueous solutions of dispersions containing 0.2 to 2% solids were shell-frozen in an acetone-Dry Ice bath. The water was removed with an NRC Type 3505-2 Dehydration Unit manufactured by the NRC Equipment Corp., Newton Highlands, Mass.

PAPER CHROMATOGRAPHY

All chromatograms were developed in a room maintained at 81°F. Qualitative and quantitative chromatography were carried out with Whatman No. 1 filter paper. For preparative work, Whatman 3MM and No. 17 filter papers were used, and were prewashed with distilled water. Quantitative and preparative chromatograms were conditioned for at least 12 hours prior to development.

Spray reagents included p-anisidine hydrochloride (104), aniline hydrogen phthalate (105), 2,3,5-triphenyltetrazolium chloride (105), and alkaline silver nitrate (106).

The following developers were employed: A, ethyl acetate–pyridine–water (8:2:1); B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); C, n-butanol–ethanol–water (4:1:5, upper) (107); D, methyl ethyl ketone–water azeotrope containing 1% ammonium hydroxide (108); and E, methyl ethyl ketone–water

azeotrope containing 1% ammonium hydroxide and saturated with sodium borate ($Na_2B_4O_7 \cdot 10\ H_2O$) (109). Developer components were mixed by volume.

For preparative chromatography, sheets of Whatman No. 17 filter paper (9 inches by 22-1/2 inches) were prepared with a support loop of Whatman No. 1 filter paper as shown in Fig. 21. After conditioning, developing, and drying, the one-inch guide strips were removed and the sugars located by appropriate spray reagents. The desired component in each sheet was located and combined to form an elution packet as shown in Fig. 22. The packets were eluted with distilled water for about 24 hours and the eluate concentrated *in vacuo* at 45°C. (31).

QUALITATIVE MICROHYDROLYSIS OF POLYSACCHARIDES (110)

To 10 mg. of hemicellulose in a 3-ml. test tube was added 0.15 ml. of 88% formic acid. The mixture was heated on the steam bath until the hemicellulose dissolved (about 30 minutes). Then 0.3 ml. of 1N sulfuric acid was added, and the mixture heated on the steam bath for 3 to 4 hours. The solution was neutralized with 30 mg. of barium carbonate and centrifuged. The supernatant liquid was chromatographed directly.

For methylated polysaccharides the procedure suggested by Croon, *et al.* (80) was employed. To 20 mg. of the methylated polysaccharide in a 3-ml. test tube was added 0.2 ml. of 72% sulfuric acid. After standing at room temperature for one hour the acid was diluted to 12% by the addition of 0.8 ml. of distilled water and held at 90°C. for 5 hours. The hydrolyzate was transferred to a 50-ml. beaker with a few milliliters of distilled water and neutralized to pH 5.5 with saturated barium hydroxide. The mixture was centrifuged, and the supernatant liquid concentrated *in vacuo* at 45°C. and chromatographed.

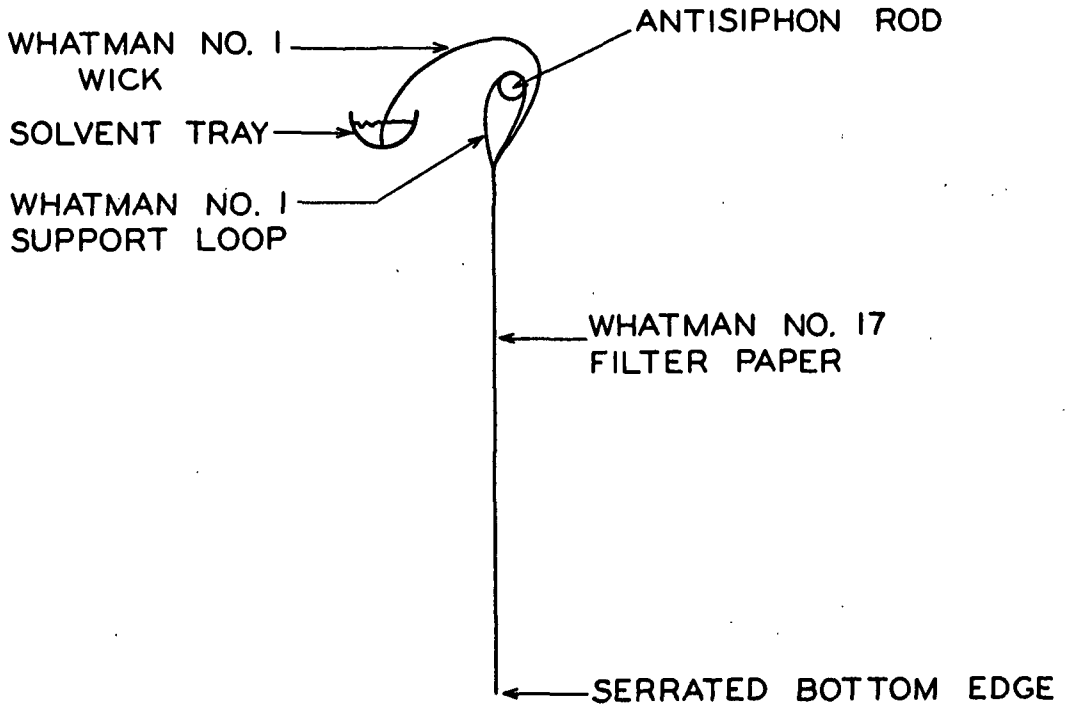


Figure 21. Preparative Chromatogram (Side View)

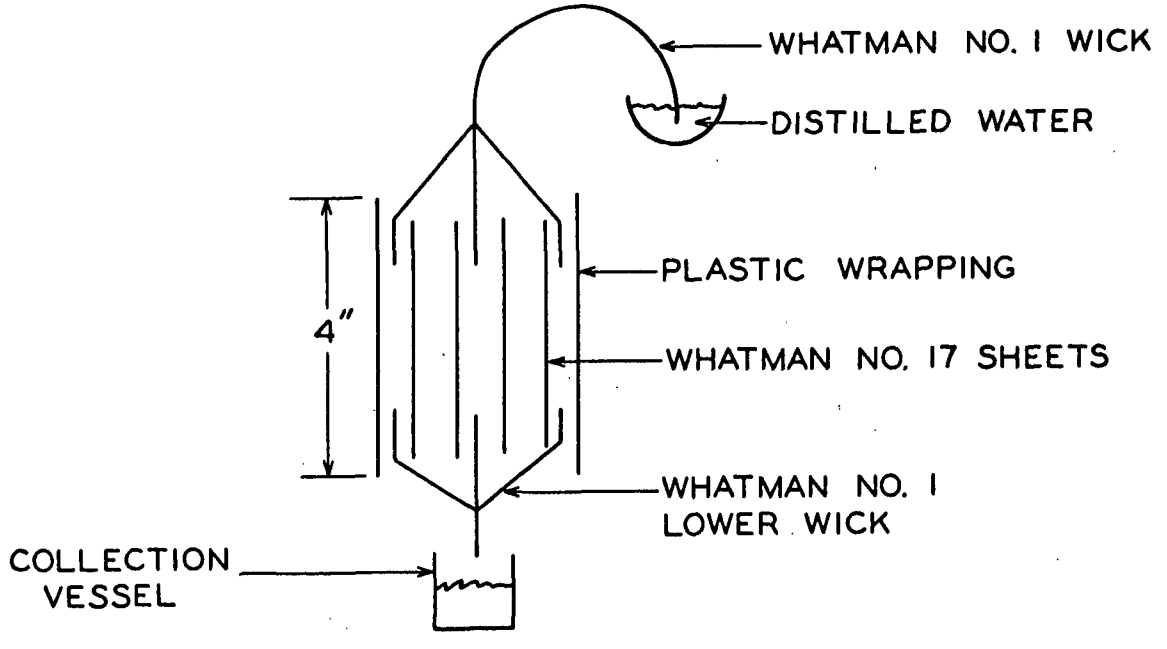


Figure 22. Elution Packets for Preparative Chromatograms (Side View)

DEMETHYLATION OF MONOSACCHARIDES (104)

An aqueous solution of the methylated sugar (about 5 mg.) was placed in a 2-ml. ampule and evaporated to dryness using a stream of dry air. One milliliter of hydrobromic acid (48%) was added and the tube sealed. After heating for 20 minutes at 90°C. the contents of the tube were transferred to a 50-ml. beaker and diluted to 10 ml. with distilled water. Small portions of silver carbonate were added until the solution was neutral to litmus. After filtering through a glass fiber filter* the solution was concentrated *in vacuo* at 45°C. and chromatographed in Solvent A.

*Glass fiber filter paper (No. 934-AH) is made by Hurlbut Paper Co. and distributed by H. Reeve Angel & Co., Clifton, N. J.

APPENDIX III

PRELIMINARY EXPERIMENTS IN THE PREPARATION OF THE O-ACETYL GLUCOMANNAN PHENYLCARBAMATE (GM-OAc-PC)

An exploratory study was made to determine the optimum conditions for preparing the GM-OAc-PC. When the GM-OAc was added to dimethylformamide a viscous gel was obtained. However, once the phenylisocyanate was added and the mixture heated on the steam bath, a clear brown solution resulted.

The ratio of dimethylformamide to GM-OAc was always 20. The results of the preliminary work are given in Table VIII.

TABLE VIII

REACTION OF GM-OAc WITH PHENYLISOCYANATE

Sample	Phenyl-isocyanate, ml./g.	Reaction Time, hr.	Yield, %	Nitrogen, %
I	2.5	5	52	7.28
IR[a]	1.7	5	22	7.53
II	3.0	6	62	7.49

[a]A portion of Sample I was given a second treatment.

The theoretical nitrogen content of the GM-OAc-PC based on a pure hexosan is 7.74%. From Table VIII it appears that the conditions used for Sample II give the best combination of high yield and high nitrogen content. Consequently, these conditions were used for the large-scale preparation of the GM-OAc-PC.

APPENDIX IV

ISOLATION AND CHARACTERIZATION OF GLAZIER'S (54) ALKALI-EXTRACTED PARAÑA PINE GLUCOMANNAN

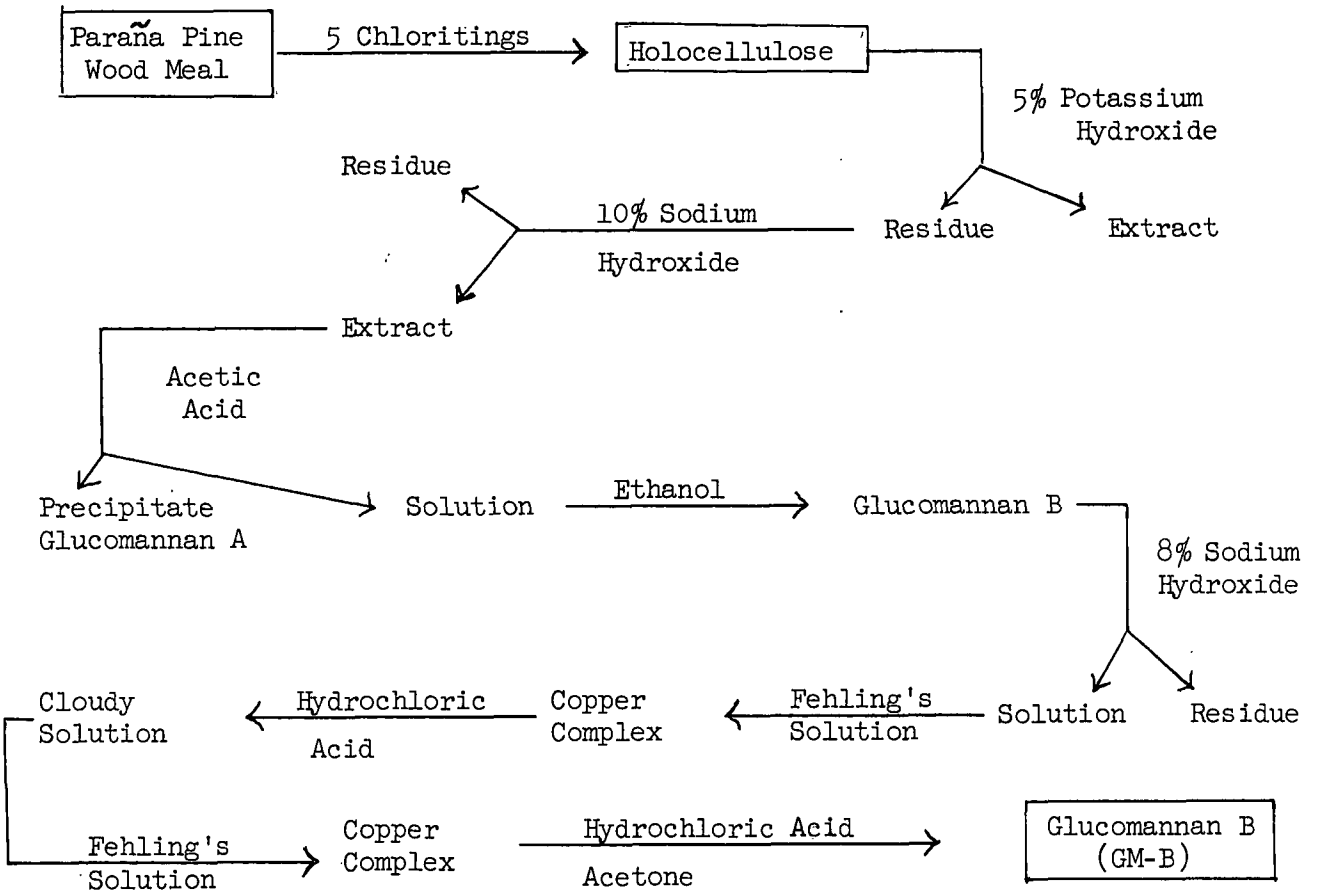


Figure 23. Flow Diagram for Isolation of Glazier's (54) Alkali-Extracted Paraña Pine Glucomannan

The glucomannan (GM-B) contained 1.8% galactan, 21.8% glucan, 56.9% mannan, 0.5% araban, 1.1% xylan, 5.8% uronic anhydride, and 1.5% sulfated ash. These values are based on the ovendry material.

APPENDIX V

ATTEMPTED FRACTIONATION OF ACETYLATED GLUCOMANNAN ON DEAE CELLULOSE

The acetylated glucomannan (5.9 g.) was dissolved in 0.01M sodium phosphate buffer (pH 5.5) and put on a DEAE* column of 5 x 40 cm. The column had been conditioned with 0.5M sodium phosphate and then washed with 0.01M sodium phosphate. The flow rate of about 60 ml. per hour was established, and 18- to 20-ml. fractions were collected automatically every 20 minutes by a Technicon fraction collector. A graded series of buffer solutions was employed including 0.01M, 0.3M, and 0.5M sodium phosphate. All buffer solutions were prepared from a 0.5M stock solution containing 0.475 mole per liter $NaH_2PO_4 \cdot H_2O$ and 0.25 mole per liter Na_2HPO_4. The column was finally washed with 0.1N sodium hydroxide. The polysaccharide content of the eluate was monitored by the phenol–sulfuric acid method (111). An aliquot (0.04 ml.) was added to a 10-ml. colorimeter tube followed by 1.0 ml. of 5% phenol solution and 5.0 ml. of concentrated sulfuric acid. The optical density was measured at 490 mμ.

Figure 24 shows that essentially three fractions were obtained. Each fraction was deionized by passing through columns of IR-120(H) and IR-45(OH). After concentration to a small volume in a circulating evaporator, the fractions were poured into five volumes of methanol and washed successively with ether and petroleum ether (30-60°C.). Fractions I and II were dissolved in water, filtered, and freeze-dried. All fractions were analyzed for sugars, and Fractions I and II for O-acetyl. The intrinsic viscosities of Fractions I and II in 1M cupriethylenediamine were also determined. The fractionation is summarized in Table IX.

*Obtained from the Brown Co., Berlin, N. H. Type 20, capacity 1.05 meq./g.

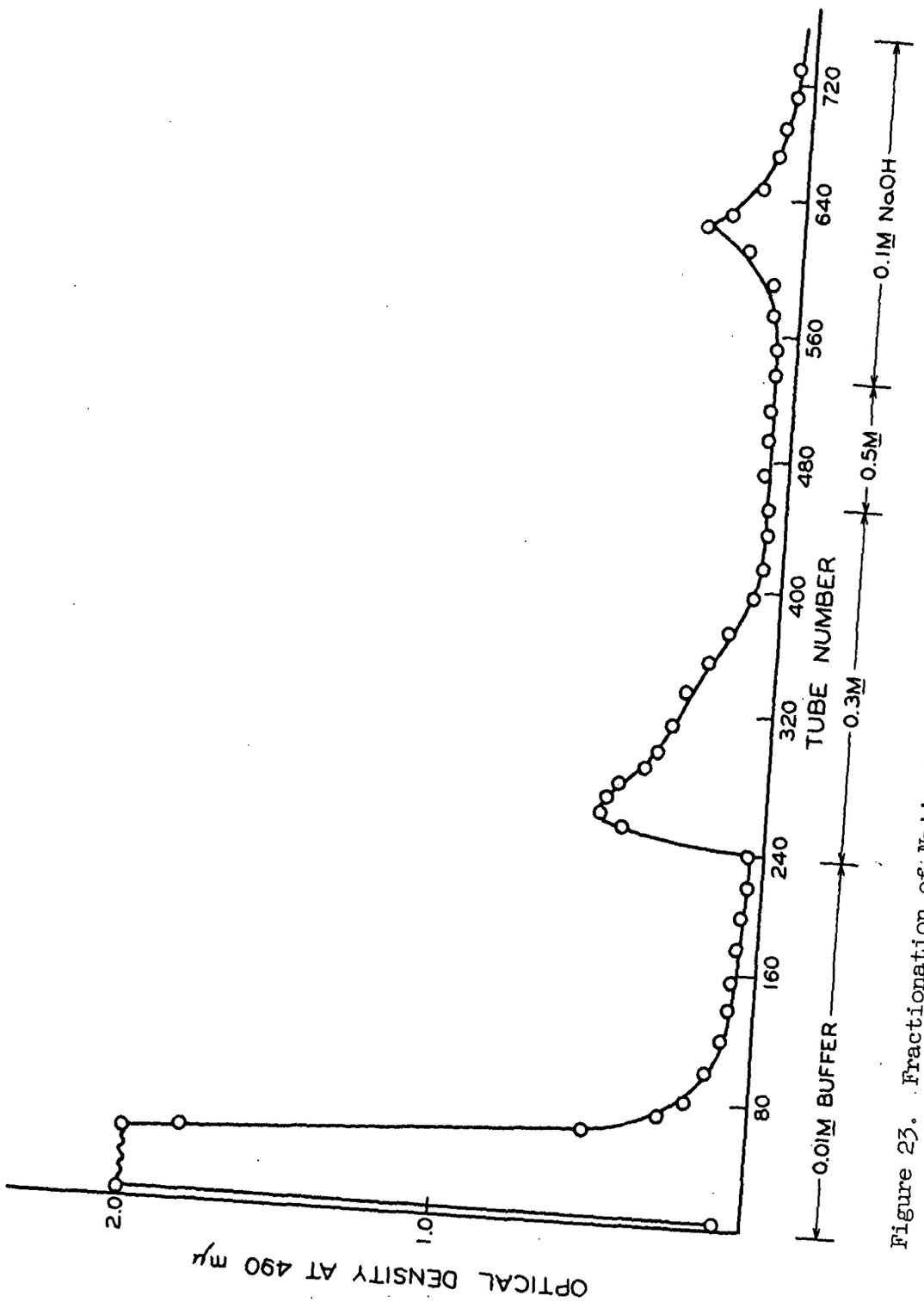


Figure 23. Fractionation of Natively Acetylated Paraná Pine Glucomannan on DEAE Cellulose

TABLE IX

SUMMARY OF DEAE FRACTIONATION OF GM-OAc

Material	Carbohydrates, %[a]					Yield, g.	O-Acetyl, %	$[\eta]$, dl./g.[b]	Ratio of Man./Gl.
	Gal.	Gl.	Man.	Ar.	Xy.				
GM-OAc	6.1	18.1	68.7	1.7	5.4	5.9	5.86	0.38	3.8
Fr. I	5.8	39.8	54.4	--	--	2.35	2.65	0.35	1.4
Fr. II	4.7	19.0	75.5	--	0.8	0.99	2.23	0.33	3.9
Fr. III	5.6	26.9	48.4	2.7	16.4	0.33	--	--	1.8

[a]Carbohydrate content adjusted to 100% of material analyzed.
[b]Intrinsic viscosity in 1M cupriethylenediamine.

In Table X the fractionation obtained in this work is compared to that of Meier (24) and Annergren, et al. (27). The most obvious difference is that this investigation shows the presence of two acetylated glucomannan fractions instead of the single fraction obtained by both Meier (24) and Annergren, et al. (27). This may be explained by the fact that a fraction cutter was employed in this work and the fractionation monitored with phenol–sulfuric acid reagent. Both Meier (24) and Annergren, et al. (27), however, eluted with arbitrary volumes of buffer and combined eluates of similar carbohydrate content. They did not monitor their fractionation in any other manner.

The second major difference is the abnormally high glucose content of Fraction I. This is believed to be caused by a glucan contaminant desorbed from the column only in the presence of the sample. Since the column was thoroughly prewashed for over four weeks with 0.5M and 0.01M buffer, it appears that the presence of the sample somehow triggered the removal of the glucan from the column. The DEAE was assumed to be in the free hydroxyl form, and was consequently saturated with phosphate buffer. Perhaps it would have been better to make sure that the DEAE was in free hydroxyl form by regeneration with sodium hydroxide.

TABLE X

COMPARISON OF ACETYLATED GLUCOMANNANS
BEFORE AND AFTER DEAE FRACTIONATION

Source	Carbohydrates, %[c]					O-Acetyl, %[b]	Ratio of Man./Gl.
	Gal.	Gl.	Man.	Ar.	Xy.		
Meier (24)							
B. F.[a]	8.4	16.5	60.8	2.8	11.5	4.51	3.7
A. F.[b]	4.8	18.0	77.2	--	--	5.95	4.2
Annergren, et al. (27)							
B. F.	4.7	17.9	68.2	2.0	7.2	5.4	3.9
A. F.	1.3	20.9	77.8	--	--	7.9	3.7
This Work							
B. F.	6.1	18.1	68.7	1.7	5.4	5.86	3.8
A. F.							
Fr. I	5.8	39.8	54.4	--	--	2.65	1.4
Fr. II	4.7	19.0	75.5	--	0.8	2.23	3.9

[a]Before fractionation.
[b]After fractionation.
[c]Carbohydrate content adjusted to 100% of material analyzed.

Evidence for a contaminant from the column was obtained from two sources. Fraction I appeared in the eluate after approximately 100 ml. of solution had been collected. This is too small a hold-up volume for a 5 x 40 cm. column. Also, a material balance showed that the recovery of glucose was 120%, while the recovery of galactose and mannose was 61 and 58%, respectively.

The O-acetyl contents of both Fractions I and II were unusually low compared to the results of Meier (24) and Annergren, et al. (27). Their

recovered polysaccharides accounted for 33.8 and 93.6% of the O-acetyl groups present in their unfractionated polymers. In this work, 24.6% of the O-acetyl groups in the acetylated glucomannan could be accounted for by Fractions I and II. Even after correcting for the glucan contaminant (28.9%), the acetyl content of Fraction I was only 3.72%. In addition, the infrared spectra of Fractions I and II showed weaker carbonyl absorption than the unfractionated acetylated glucomannan. These results indicate that some deacetylation may have occurred during the fractionation.

The results of the DEAE fractionation are inconclusive and do not add to the body of this thesis. For this reason the experiment is presented as an appendix. It is obvious that more work needs to be done in this general area of fractionation of polysaccharides. Perhaps the use of Sephadex columns (99) will obviate some of the difficulties encountered with DEAE cellulose.